Some Ecological Factors Affecting the Input and Population Levels of Total and Faecal Coliforms and *Salmonella* in Twelve Mile Creek, Lake Ontario and Sewage Waters Near St. Catharines, Ontario.

J. M. Roth, B. A.

Department of Biology

(Submitted in partial fulfillment of
the requirements for the degree of
Master of Science)

B R O C K U N I V E R S I T Y

St. Catharines, Ontario.

June 1975

ABSTRACT

Roth, James Milton. M.Sc., Brock University, June, 1975.

Some Ecological Factors Affecting the Input and Population Levels of Total and Faecal Coliforms and Salmonella in Twelve Mile Creek, Lake Ontario and Sewage Waters Near St. Catharines, Ontario. Supervisor: Dr. M. Helder.

The present study was undertaken to investigate the role of some ecological factors on sewage-borne bacteria in waters near St. Catharines, Ontario. Total and faecal coliform levels and the presence of Salmonella were monitored for a period of a year along with determination of temperature, pH, dissolved oxygen, total dissolved solids, nitrate N, total phosphate P and ammonium N. Bacteriological tests for coliform analysis were done according to APHA Standard Methods by the membrane filtration technique. The grab sampling technique was employed for all sampling.

Four sample sites were chosen in the Port Dalhousie beach area to determine what bacteriological or physical relationship the sites had to each other. The sample sites chosen were the sewage inflow to and the effluent from the St. Catharines (Port Dalhousie) Pollution Control Plant, Twelve Mile Creek below the sewage outfall and Lake Ontario at the Lakeside Park beach. The sewage outfall was located in Twelve Mile Creek, approximately 80 meters from the creek junction with the beach and piers on Lake Ontario. Twelve Mile Creek normally carried a large volume of water from the Welland Canal which was diverted through the DeCew Generating Station located on the Niagara Escarpment. An additional sample site, which was thought to be free of industrial wastes, was chosen at Twenty Mile Creek, also in the Niagara Region of Ontario.

There were marked variations in bacterial numbers at each site and between each site, but trends to lower numbers were noted from the sewage inflow to Lake Ontario. Better correlations were noted between total and faecal coliform population levels and total phosphate P and ammonium N in Twenty Mile Creek. Other correlations were observed for other sample stations, however, these results also appeared to be random in nature. Salmonella isolations occurred more frequently during the winter and spring months when water temperatures were minimal at all sample stations except the sewage inflow. The frequency of Salmonella isolations appeared to be related to increased levels of total and faecal coliforms in the sewage effluent. However, no clear relationships were established in the other sample stations. Due to the presence of Salmonella and high levels of total and faecal coliform indicator organisms, the sanitary quality of Lake Ontario and Twelve Mile Creek at the sample sites seemed to be impaired over the major portion of the study period.

ACKNOWLEDGMENT

Much gratitude is extended to Dr. M. Helder who agreed to undertake the position of Supervisor of the thesis.

Many thanks to Dr. A. J. S. Ball for guidance in statistical methodology and organization.

The author is very grateful to Dr. E. A. Belle and the Ontario Ministry of Health for allowing full use of the laboratory facilities.

The author is indebted to Mr. J. Burnside, Chief Engineer, St. Catharines Pollution Control Plant, for allowing the study of the sewage treatment system.

Thanks to Ms. L. McNab and Mr. J. Sedgwick for aiding in the endless task of bacteriology.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE NO. PAGE NO.

LIST OF PLATES

I INTRODUCTION

The diversity of bacterial types that may be found in an aquatic community is impressive. These organisms can be subdivided into two major groups in terms of energy sources utilized for metabolism and growth; autotrophic and heterotrophic bacteria. The autotrophic bacteria are able to derive energy for metabolism from other than preformed organic compounds. These alternate sources include sunlight and reduced inorganic compounds. By contrast, the heterotrophic bacteria must obtain energy from organic sources and, as a group, have widely varying nutritional requirements. Along with algae and higher aquatic plants, autotrophic bacteria function as net producers in an aquatic community, while the heterotrophic bacteria are organic degraders and comprise the largest group of true bacteria both in numbers and diversity (Kuznetsov, 1968).

The bacteria present in natural waters may be classed either as resident aquatic organisms or as non-aquatic, immigrant bacteria (Park, 1972). Resident bacteria are those organisms which occur naturally in water and can actively grow and compete for nutrients in a nutrient-low environment. Immigrant bacteria are normally found only in low numbers in unpolluted natural waters. They have been carried or placed into an aquatic environment where they are usually not adapted to obtain sufficient nutrition. Not only are high quality surface waters low in many inorganic nutrients, but they can also lack many organic compounds important to immigrant organisms either as nutrients or growth factors. Physical conditions such as pH, oxygen content or temperature ranges may also be unfavorable to the survival of non-aquatic bacteria. Depending on the

bacterial types, occasional growth of non-aquatic organisms can be observed in natural waters. During such a phenomenon, the water in question is usually found to contain higher than normal levels of nutrients (Coleman, Campbell, Cook and Westlake, 1974; Hendricks and Morrison, 1967). After the natural population balance has been upset by the introduction of various nutrients and immigrant bacteria, the water gradually is cleansed by nutrient removal, oxygenation and the effects of sunlight (Leclerc, 1963). Immigrants are then again replaced by the natural bacterial populations, but this may take a considerable length of time depending upon the volume of organic pollution and stream or lake physiographics (Coleman et al., 1974; Deaner and Kerri, 1969).

Resident aquatic bacteria may use either aerobic or anaerobic metabolic pathways, depending upon the genus or species. However, the anaerobes will not be discussed beyond mention of the anaerobic photosynthetic processes of the purple and green bacteria. Both groups are Gram-negative, motile rods and can be distinguished from other Gram-negative organisms by their chlorophyllous pigments. The Gram-negative aerobic chemoautotrophs lack chlorophyllous pigments and obtain energy for metabolism from the oxidation of some reduced inorganic compounds and can use CO_2 as their sole carbon source. The chemoautotrophs can be subdivided into groups according to their respective inorganic energy source. Not all organisms in these groups are obligate chemoautotrophs and some, therefore, are also capable of using various organic compounds as sources of energy when they are available. In that case they would be growing heterotrophically. The largest group of chemoautotrophs are the hydrogen bacteria. These are Gram-negative motile rods, some of which belong to the genus Pseudomonas.

They are able to use H_2 as an energy source. Another group, the nitrifying bacteria, are important to the overall cycle of nitrogen in the biosphere. Their roles include the oxidation of nitrite to nitrate. These include the Gram-negative, motile rods Nitrosomonas and Nitrobacter respectively, both of which are commonly found in water. Thiobaccillus, also a Gram-negative rod, is the single genus which is responsible for the chemoautotrophic oxidation of sulfur. The last group of important aquatic chemoautotrophs is the iron bacteria which grow at the expense of reduced iron oxide. The genus Gallionella is aerobic like many iron bacteria and it is a Gram-negative rod.

The bulk of aquatic bacteria are aerobic, Gram-negative rods and they are heterotrophs. The diversity of substrate and of other environmental requirements within this group is exceedingly wide. Some groups of commonly occurring bacteria in fresh water and soil are the pseudomonads, Azotobacter, Spirillum, Sphaerotilus and Caulobacter (Guthrie, Cherry and Ferebee, 1974). The pseudomonads are able to utilize a wide variety of organic compounds and a few representatives are able to attack some of the more exotic organic compounds present in water. Some members which are denitrifiers, can grow facultatively as anaerobes with nitrates acting as a terminal electron acceptor. The Azotobacter group, specifically A. vinelandi and A. agilis, can be distinguished from other Gram-negative heterotrophs by their ability to fix N_2. The Sphaerotilus group of bacteria can be motile or they can become sessile, forming mucilagenous sheaths about chains of cells. Sphaerotilus natans grows abundantly in sewage polluted streams and is often found attached to underwater stones and plants. Other heterotrophic organisms typically found in unpolluted waters include the Gram-positive organisms Bacillus, Sarcina, Staphylococcus and

Micrococcus and the Gram-negative organisms Chromobacterium, Enterobacter, Flavobacterium, Serratia, Brevibacterium and Achromobacter (Guthrie et al., 1974; Cherry, Guthrie and Harvey, 1974).

Immigrant bacteria in natural waters generally belong to the heterotrophic group of organisms. The varieties and sources of these contaminants are almost limitless. The enteric organisms are one of the largest subgroups of Gram-negative, non-photosynthetic, true bacteria that can be found regularly in most surface waters. These organisms have rod-shaped cells, either straight or curved, not exceeding 2.0μ in width, and can be either permanently immotile or motile depending on the species (Bergey, 1974). The enteric bacteria are characterized by their ability to ferment carbohydrates anaerobically. However, they will grow under aerobic conditions as well. This distinguishes them from other Gram-negative bacteria. Most enteric bacteria can use a variety of simple organic compounds as energy-yielding substrates and many species have basic minimal growth requirements. The major genera comprising the Enterobacteriaceae (enteric organisms) are: Escherichia, Salmonella, Shigella, Klebsiella, Enterobacter, Serratia, Edwardsiella, Proteus, Providencia and Pectobacterium (Edwards and Ewing, 1972).

Sewage is probably the major source of enteric bacteria and other organic constituants polluting natural waters, particularly near urban centers. Human faeces in the form of raw sewage contain high levels of enteric bacteria, some of which may be pathogenic (Brezenski and Russomanno, 1968; Coleman, Campbell, Cook and Westlake, 1974). Similarly, unchlorinated sewage effluent can carry large numbers of enteric bacteria, although adequate chlorination is effective in eliminating certain pathogens (McCoy, 1963;

Grunnet and Nielsen, 1969; Brezenski, Russomanno and DeFalco, 1965). Evans, Geldreich, Weibel and Robeck (1968) were able to isolate pathogenic enteric bacteria from urban stormwater. Similarly, Geldreich, Best, Kenner and Van Donsel (1968) and Van Donsel, Geldreich and Clarke (1967) related faecal contamination of soil to levels of enteric organisms present in urban stormwater run-off. Enteric bacterial levels in streams and lakes are generally lower in rural areas than near urban centers; however, cattle feedlot run-off, livestock pastures and drainage from flood irrigation can contribute to enteric organism levels (Miner, Fina and Piatt, 1967; Petersen and Boring, 1960). Wild animal populations in isolated watersheds also may contribute significant levels of enteric organisms. Fair and Morrison (1967) recovered enteric pathogens from high quality surface waters. They stated that the species analysis was suggestive of wild animal sources. In a study of a mountain watershed in Montana, Stuart, Bissonnette, Goodrich and Walter (1971) attributed increased levels of enteric bacteria in water to wild animal sources. Once the area was closed to human use and the wild animals had returned, levels of enteric bacteria increased. Forest environments and farm produce also might make some contribution to enteric bacterial levels in water. Duncan and Razzell (1972) identified many enteric bacterial types from both sources and found some biotypes which are normally faecal in origin. Not all members of the enteric group are inhabitants of the intestinal tract; some have a different ecology. Some members of the Aerobacter (grouped in the Enterobacter genus by Edwards and Ewing, 1972), Serratia and Proteus genera are primarily soil and water organisms, while Erwinia can be a plant pathogen (Stanier, Doudoroff and Adelberg, 1970). However, these organisms are classed as members of the enteric group for their ability to ferment carbohydrates.

Some members of the Enterobacteriaceae can be enteric pathogens and these include: Salmonella, Shigella, and several bioserotypes of Escherichia (Edwards and Ewing, 1972). Their presence in drinking water and recreational water is of sanitary concern. Because pathogens may be difficult to isolate, it is often considered sufficient to isolate certain non-pathogenic organisms which also occur in the intestinal tract of man and other warm-blooded animals. These are termed organisms indicative of faecal contamination. One group of organisms considered to be particularly useful for indicating sewage pollution is the coliform group. These organisms possess the common characteristics of the other Enterobacteriaceae, but, in addition, are able to ferment lactose with gas formation within 48 hours at 35C (STANDARD METHODS, 13th ed.). Even within this group, however, some isolates can be found with a non-faecal ecology. Thus a more restricted definition of organisms with a faecal origin is necessary. This restricted group is called the faecal coliform group. They are identified by their ability to produce gas from a lactose medium at 44.5C within 24 hours (STANDARD METHODS, 13th ed.). Geldreich, Best, Kenner and Van Donsel (1968) found that a large percentage of the coliform organisms originating from the intestinal tract conformed to the faecal group. The faecal coliforms have been found to belong to much the same genera as some non-faecal types. Duncan and Razzell (1972) identified faecal coliforms from the genera Escherichia, Klebsiella and Enterobacter in forest and farm produce environments. In addition, Dutka, Bell, Colins and Popplow (1969), using the biochemical classification scheme proposed by Ewing and Edwards (1960), identified these organisms, Enterobacter, Klebsiella, Escherichia and Citrobacter, as the dominant genera in pulp and papermill

wastes in Rainey River. It is, therefore, the point of origin and the physiology which distinguishes faecal coliforms, not their taxonomy, as such.

Other bacteria, such as the faecal streptococci (STANDARD METHODS, 13th ed.) and Salmonella (Cherry, Hanks, Thomason, Murlin, Biddle and Croom, 1972), have been recommended for use as sanitary indicators. Moreover, Dutka, Chau and Coburn (1974) have considered the potential value of faecal sterols as indicators of faecal pollution. They maintained that certain faecal sterols might be useful in differentiating between industrial and faecal sources of contamination. They also thought that faecal sterols could be useful as indicators of the efficiency of various sewage treatment processes, as indicators of accumulative organic compounds (e.g. estrogens) and as indicators of potential pathogens such as enterovirus which are known to survive sewage treatment processes. The potential of these compounds for use as indicators definitely warrants further consideration.

Several factors control levels of indicator organisms in natural water systems. The first is input of indicators via pollution from sewage, stormwater run-off, industrial organic wastes and feedlot run-off sources. Input is probably the single most important factor controlling indicator levels. The concentration of organisms in the inflow and the volume of sewage inflow compared to the volume in the receiving waters, affects concentrations of indicators. During periods of heavy rain, levels of indicator organisms in water sharply increase as they are washed from the surrounding watershed (Geldreich et al., 1968). Continued precipitation beyond this point will dilute bacterial levels in water. Turbulence of receiving

waters may also influence levels of indicators by producing uneven distribution. In addition, resuspension of bacteria which have accumulated in bottom silt and mud can contribute to indicator and pathogenic bacteria levels in overlying waters (Van Donsel and Geldreich, 1971).

The third major factor which influences levels of indicator organisms is the ability of certain types of coliforms to regrow in nutrient rich waters. Coleman et al. (1974) established that small amounts of crude sewage introduced into the North Saskatchewan River from small hamlets did little more than provide nutrients which enhanced growth of the natural aquatic flora. At the same time, there were no significant increases of faecal organisms in the upper reaches of the river. However, large amounts of nutrients and an innoculum of enteric bacteria supplied by the large urban center of Edmonton did enhance regrowth of E. coli. Moreover, Dutka et al. (1969) observed aftergrowth of some coliform types found in pulp and papermill processes in Rainey River. Evans et al. (1968) found certain coliform organisms were able to regrow in dechlorinated stormwater which contained nutrient levels which ranged between 1-5 mg/l organic nitrogen and 0.7-8.1 mg/l total phosphate. In addition, they observed some slight regrowth of faecal coliform types. Hendricks and Morrison (1967) established that some Klebsiella, Arizona, Salmonella and Shigella were able to grow in dialysis sack culture located downstream from a sewage effluent outfall in the Poudre River, Colorado. Nutrient levels averaged 1.1 mg/l ammonium N, 2.0 mg/l phosphate, 0.3 mg/l glucose, 1.0 mg/l hexose and 17.0 mg/l protein. In contrast, Deaner and Kerri (1969) observed no regrowth of faecal coliforms from a sewage outfall into a relatively low nutrient stream. They concluded that the short travel time of sewage

laden waters over the study area and river physiographics may have influenced the observed lack of regrowth.

The last major factor controlling levels of indicators is their death-rate. The majority of indicator bacteria in a good-quality surface water environment are probably dying. The slower the death-rate, however, the higher will be the numbers detected and the larger the area over which they will be dispersed. Nutrient levels in water can provide for energy maintenance, and if present in high concentrations, may provide for growth. Wright and Hobbie (1966) determined that 1-10 mg/l glucose and acetate were present in many high quality natural waters. Threshold levels of glucose for growth of E. coli have been found to be 5 mg/l, while lower levels can maintain energy levels (McGrew and Mallette, 1961). Hendricks and Morrison (1967) stated that most moderately polluted waters contain more than threshold levels of glucose. Using dialysis sack culture methods, they found that some enteric bacteria (e.g. E. coli, Enterobacter aerogenes and Salmonella senftenberg) are able to grow in various media such as river bottom extract, sewage effluent receiving waters and high quality river water. Other researchers have been able to establish that nutrients provided by vegetable process waters, pulp and papermill effluent or sewage effluent can allow for some enteric organisms to survive and grow (Gallagher and Spino, 1968; Dutka et al., 1969; Coleman et al., 1974, respectively). Inorganic nutrients can also have a subtle influence on survival of coliform bacteria. Membrane filter chamber studies by McFeters and Stuart (1972) have shown that increased inorganic constituents such as total hardness, Ca, Mg, Na and K contributed to increased survival of non-faecal and faecal coliforms. Brasfield (1972), using regression analysis, was able to correlate total coliform levels

to bicarbonate levels which have an important effect on pH. She also found that chlorides were negatively correlated to total coliform levels. McFeters and Stuart (1972) also monitored pH and conductivity and found that the pH range of 5.5-7.5 was optimal for coliform survival. Coleman et al. (1974) studied some physical parameters of the Saskatchewan River and were able to demonstrate a negative correlation between pH (above 7.5) and E. coli from the alkaline headwaters to the more neutral lower reaches. They also established that a positive correlation existed between temperature and E. coli levels over the same course. These probably reflected the increased sewage loading of the river as it gradually warmed along its course towards Edmonton. However, Post (1969) found a negative correlation between both total and faecal coliform bacteria and temperature in a sewage pond, suggesting that cooler water temperature influenced coliform survival. Similarly, Geldreich et al. (1968) noted that both faecal coliforms and Salmonella persisted longer in stormwater during the winter below 10C than during the summer when water temperatures approached 20C. McFeters and Stuart (1972) found faecal coliform survival was inversely related to temperature below 15C. An interesting aspect of temperature effects on bacterial populations in polluted and non-polluted waters has been explored by Guthrie, Cherry and Ferebee (1974) and Cherry, Guthrie and Harvey (1974). They concluded that a 3-4C increase of water temperature above the ambient resulted in increases in numbers of aquatic bacteria, including levels of enteric organisms. By comparison, a 5-10C increase of temperature resulted in further increases in raw numbers, but bacterial diversity decreased. Oxygen content also can influence the survival of some bacteria. Allen, Pasley and Pierce (1952) found that E. coli appeared to survive longer in the presence of oxygen than under anaerobic conditions.

Various biotic factors can contribute to the death-rate of coliform indicator bacteria. Some organisms are able to produce substances which are toxic to bacteria. Matusiak, Chróst and Krzywicka (1971) were able to show that Chlorella vulgaris cultures inhibited the growth of some Gram-positive organisms, while most Gram-negative organisms were resistant. The commercially important soil actinomycete Streptomyces produces various antibiotics which can have a lethal effect on many organisms in soil. It is not too unreasonable to expect some aquatic actinomycetes to have a similar effect on aquatic bacteria. Another factor leading to decreased levels of indicator bacteria is grazing. Some soil amoebae, and the ciliates Colpidium and Colpoda, can utilize bacteria as food sources, both in soil and water (Purdy and Butterfield, 1917; Singh, 1945; Coler and Gunner, 1969). Coler and Gunner (1969) established that Colpoda, a soil ciliate, preferred the coliform, E. coli and Enterobacter aerogenes to the naturally occurring soil bacterium Arthobacter, which has a toxic cytoplasmic principle. Thus preferential grazing can cause one group of organisms to decline faster than others. Further studies might reveal whether preference for immigrant bacteria is general among aquatic protozoa. Bacteriophage is another biotic factor which influences the death-rate of certain enteric bacteria (Joyce and Weiser, 1967; Hendricks, 1974).

The comparative survival of enteric organisms in water and the reliability of faecal and non-faecal coliform bacteria as indicators of enteric pollution have been the subject of many studies over the past ten years. McFeters, Bissonnette, Jeseski, Thompson and Stuart (1974), using dialysis chamber techniques, demonstrated that faecal coliform bacteria have similar survival characteristics to some Salmonella in well water.

Geldreich (1970), and Van Donsel and Geldreich (1971), also found that Salmonella had similar survival characteristics to faecal coliforms. Van Donsel and Geldreich (1971) were able to establish a prediction model for Salmonella presence knowing levels of faecal coliforms. In general, they suggested that low faecal coliform indices corresponded to few or no Salmonella. They also suggested that when low coliform levels were observed, along with the presence of Salmonella, the sources of each might be cattle or wild animals. Similarly, Slanetz, Bartley and Metcalf (1964) established a relationship between faecal coliforms and Salmonella in seawater, indicating the utility of using faecal coliforms as indices of sewage pollution. By contrast, some studies report conflicting survival and correlation results between faecal coliforms and Salmonella. Gallagher and Spino (1968) noted little correlation between levels of total and faecal coliforms and Salmonella. Other studies have revealed the presence of Salmonella in drinking water supplies when coliforms levels were very low or nonexistant (Greenburg and Ongerth, 1966; Seligmann and Reitler, 1965). Seligmann and Reitler (1965) also noted that Salmonella can be more resistant to dessication than E. coli, and thus, are able to contaminate water for a longer period of time from air-borne dust. Reports of enteric bacterial contamination of high quality surface waters cast some doubt on the probable existance of uncontaminated surface waters (Fair and Morrison, 1967; Bissonnette, Stuart, Goodrich and Walter, 1970; Petersen and Boring, 1960; Cherry, Hanks, Thomason, Murlin, Biddle and Croom, 1972). Because of the ubiquitous nature of Salmonella and occurrences in conjunction with other enteric organisms, Cherry et al. (1972) have suggested using the presence of Salmonella as a supplementary index of faecal pollution. They found flourescent antibody

staining techniques to have a much greater sensitivity than standard culture methods.

The consequence of the presence of enteric indicator organisms and potential enteric pathogens in recreational waters is not fully understood. The authors of STANDARD METHODS (13th ed.) remark that more epidemiological evidence on the potential hazard of these organisms in recreational waters is necessary before conclusions can be made. Geldreich (1971) discussed results of a bacteriological study of Buffalo Lake. He concluded that, if necessary, restrictions on use during peak contamination times should be instituted in addition to clean up of sewage sources. Claudon et al. (1971) regularly isolated Salmonella from Lake Mendota which was used extensively for recreation. They emphasized the potential hazard of Salmonella in recreational waters. Presently, the acceptable limits for faecal coliform levels in primary contact recreational waters are 200 faecal coliform organisms per 100 ml (Geldreich, 1970). However, the Ontario Government policy for rejection of contaminated waters in "Guidelines and Criteria for Water Quality Management in Ontario"[1] is 100 faecal coliform organisms per 100 ml. The guidelines further state that water should be free of pathogenic organisms. These restrictions would seem to eliminate from use many water recreational areas in southern Ontario. Discrepancies of bacteriological criteria for recreational waters do indicate that additional information on the ecology of the enteric organisms would be useful.

The study area in the Niagara Region included sample stations at Lake Ontario, Twelve Mile Creek and the St. Catharines sewage treatment

---

[1]Ontario Ministry of the Environment. February, 1973. Guidelines and Criteria for Water Quality Management in Ontario. p 24.

plant in the Port Dalhousie area. For comparative purposes, a sample station at Twenty Mile Creek, also in the Niagara Region, was chosen because the creek was relatively free of industrial wastes and received no known large municipal sewage discharge. An ecological study in this area could possibly relate total and faecal coliform levels to Salmonella presence and also relate coliform levels to several environmental factors. The study was designed to investigate the following:

(1) Could the population levels of total and faecal coliform bacteria be correlated to the physical factors; temperature, total dissolved oxygen, pH and/or the nutritional factors; nitrate N, phosphate P or ammonium N?

(2) What, if any, differences were observed for the appearance of Salmonella during the study period? Were these differences related to season or temperature?

(3) Did the presence of Salmonella correspond with high levels of coliform organisms?

(4) What implications do these findings have on the suitability of the study area for recreation?

II MATERIALS AND METHODS

A - SAMPLE AREAS

Port Dalhousie on Lake Ontario was chosen as the study area because the area is used extensively for recreational purposes despite its past history of beach posting dates (during the summer of 1966 and again the first week of August 1972). This area is of particular interest because the St. Catharines Pollution Control Plant is located near the beach area (Figure 1). The pollution aspects of the area are interesting as Port Dalhousie Beach at Lake Ontario receives the "bacteriological load" of Twelve Mile Creek which bissects the beach area. Twelve Mile Creek carries much of the storm drainage, industrial wastes and raw and treated sewage from the city of St. Catharines which has about 100,000 inhabitants. In addition, it extends beyond the urban area and collects agricultural run-off.

During the sampling period, the Pollution Control Plant employed only primary treatment, and the chlorination was confined to May, June, July, August and a portion of September (personal communication from Mr. J. Burnsides, Chief Engineer, St. Catharines Pollution Control Plant). Samples were collected from the sewage inflow and effluent in order to determine the effects (if any) of the primary sewage treatment and chlorination. Indicated in Figure 1 is the overflow bypass sewer which carries storm-water overflow from the sewage inflow culvert, around the sewage treatment process, directly into the sewage effluent culvert. The significance of the bypass sewer lies in the fact that much stormwater and untreated sewage flow directly into Twelve Mile Creek, receiving no treatment during overflow

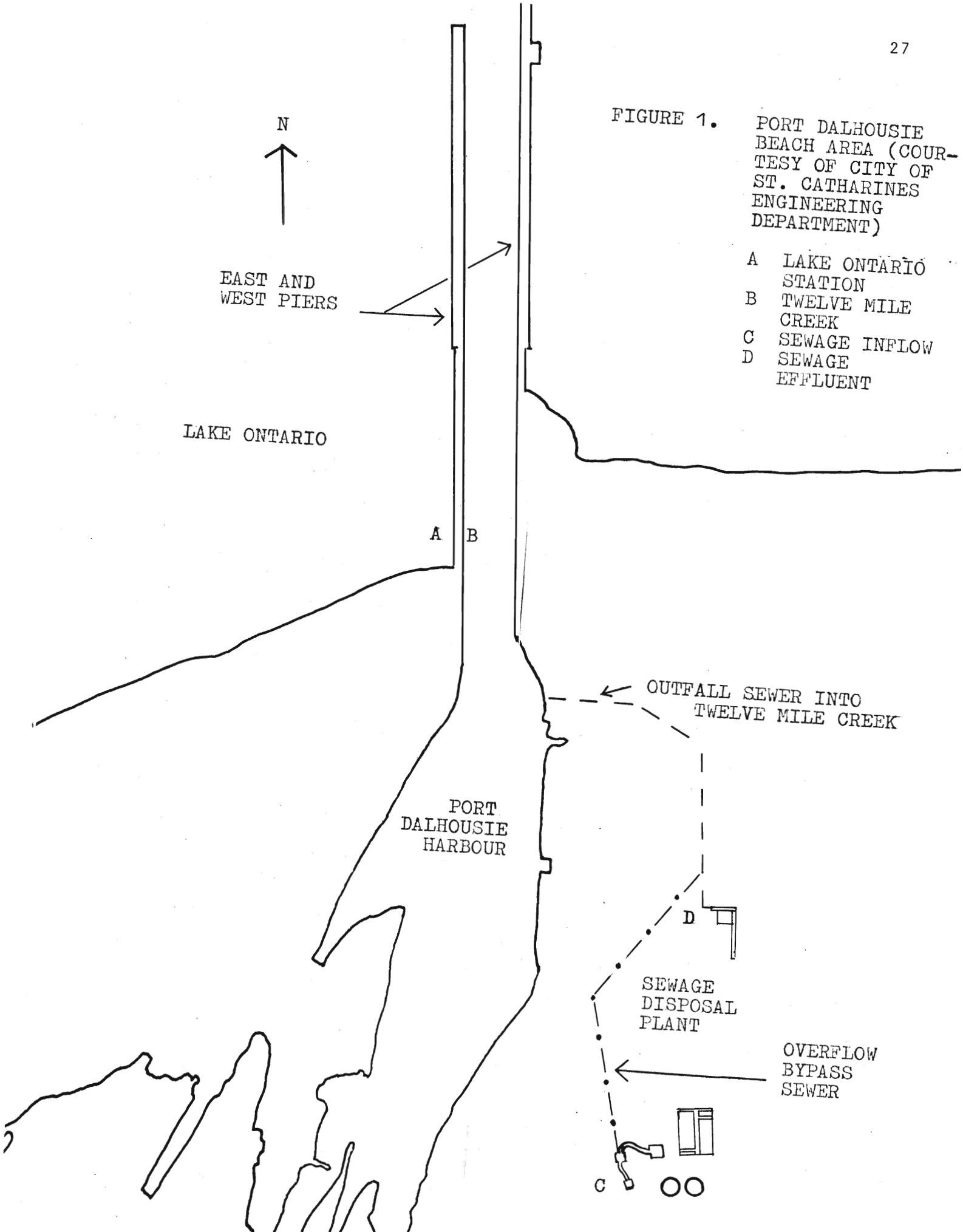


FIGURE 1. PORT DALHOUSIE BEACH AREA (COURTESY OF CITY OF ST. CATHARINES ENGINEERING DEPARTMENT)

A LAKE ONTARIO STATION
B TWELVE MILE CREEK
C SEWAGE INFLOW
D SEWAGE EFFLUENT

periods. The outflow of the treated sewage in Twelve Mile Creek is just eighty meters from the intersection of the Port Dalhousie Beach and the piers of the creek (Plate I) and the outflow pipe lies forty meters from the shore of Twelve Mile Creek, under the water surface. The east and west piers are remnants of the "old" Welland Canal and extend approximately twelve hundred meters into Lake Ontario (Figure 1).

Twenty Mile Creek (located in Lincoln County, Ontario) was also chosen as a sample site with the hope that additional information might be gathered to demonstrate the effects and contributions of agricultural run-off to Salmonella and coliform populations in a stream relatively unaffected by industrial wastes.

B - COLLECTION OF WATER SAMPLES

Water samples from five stations in Port Dalhousie and Lincoln County, Ontario, were analyzed for the presence of Salmonella and for levels of total and faecal coliform bacteria. Each station was sampled three or four times a month from August 14, 1971 to August 3, 1972. Sample stations A, B, C and D are indicated in Figure 1 and represent the Lake Ontario Beach, Twelve Mile Creek, sewage inflow and sewage effluent stations respectively.

Each week the samples were collected in the mornings beginning with sample A taken at 7:15-7:30 a.m. The other samples were collected in order (B, C and D) with five to ten minutes elapsing between each station. The Twenty Mile Creek station (E) was sampled on a subsequent morning. After collection, the samples were taken directly to the laboratory within an approximate 45 minute period between sampling and processing. During cooler weather, no special effort was necessary to maintain low water temperatures, however, during warm weather, all samples were transported in a cooler.

PLATE I - ST. CATHARINES POLLUTION CONTROL PLANT SEWAGE OUTFALL TO TWELVE MILE CREEK.


CAUTION
OUTFALL SEWER
STRUCTURE 125 FEET
CITY OF ST. CATHARINES

1 - LAKE ONTARIO BEACH STATION

Sample A was collected from Lake Ontario near the Port Dalhousie Beach approximately thirty meters from the shoreline off the western pier (Plate II). The depth of the water at this station ranged from one to one and one-half meters depending upon seasonal factors. The sample was collected from the surface to avoid collecting large amounts of silt disturbed by wave action. Depending on turbulence, the water varied from clear to turbid. Normally there was a minimum of littoral vegetation except for some green and blue-green algae washed ashore during the latter part of the summer. During the spring of 1972 many dead fish (alewives) were washed ashore. However, all traces were removed prior to the bathing season. Inspection of the beach for several hundred meters on either side of the piers revealed no drain culverts or sewage outfalls except for drainage reaching the beach indirectly from Twelve Mile Creek (Plate III).

2 - TWELVE MILE CREEK

Sample station B was also located thirty meters from the beach off the western pier of Twelve Mile Creek. The sample was collected from the creek side of the pier (Plate IV). The water current at the sample point averaged one-half meter per second and the depth ranged from five to six meters. The sample at this station was collected near the surface. The creek remained very turbid throughout the year and supported little vegetation except for Cladophora and some sewage fungus present on submerged portions of the pier.

3 - SEWAGE INFLOW

Sample station C was located in the St. Catharines Pollution Control Plant (Plate V) at the raw sewage inflow culvert (Plate VI). Water

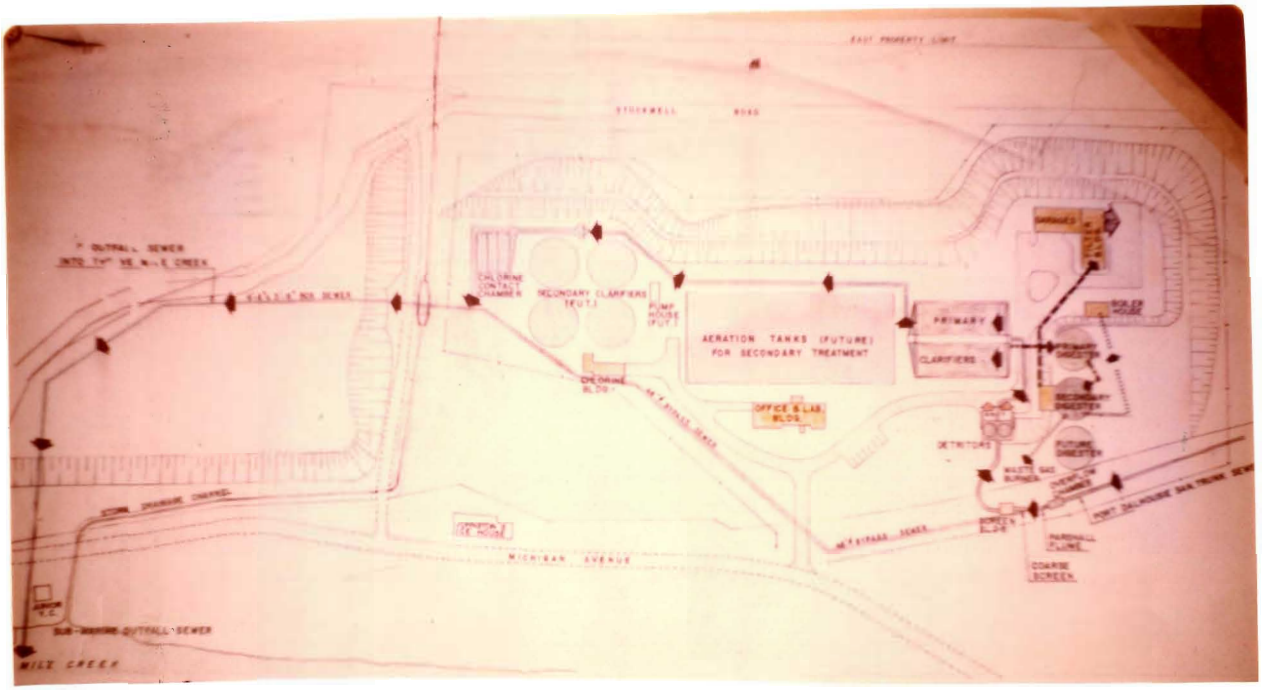
PLATE II - PORT DALHOUSIE BEACH SAMPLE STATION.

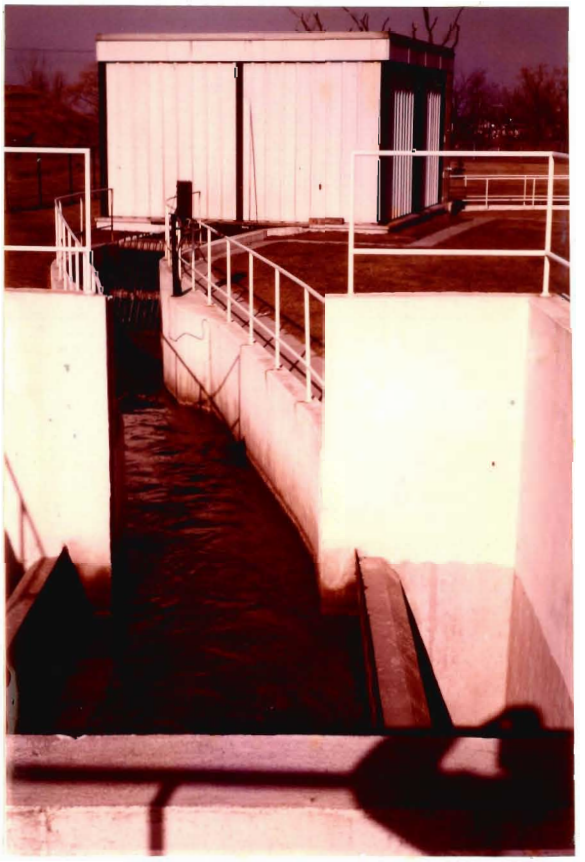
PLATE III - PORT DALHOUSIE BEACH.

PLATE IV - TWELVE MILE CREEK SAMPLE STATION.

PLATE V - ST. CATHARINES POLLUTION CONTROL PLANT.

PLATE VI - ST. CATHARINES POLLUTION CONTROL PLANT SEWAGE INFLOW SAMPLE STATION.


EAST PROPERTY LIMIT
STOCKWELL ROAD
CHLORINE CONTACT CHAMBER
SECONDARY CLARIFIERS (PLANT)
PUMP HOUSE (PLANT)
AERATION TANKS (FUTURE) FOR SECONDARY TREATMENT
PRIMARY CLARIFIERS
OFFICE & LAB BLDG.
DIGESTERS
MICHIGAN AVENUE
COARSE SCREEN
MILL CREEK



current in the culvert was several meters per second, while the depth varied from one-half to two meters. Again, the sample was collected at the surface. The sewage in the open culvert, as it entered the plant, was exposed to sunlight for no longer than a few seconds.

4 - SEWAGE EFFLUENT

Sample station D was located at the sewage outfall culvert downstream from the chlorination chamber (Plate VII). When heavy precipitation occurred in the summer, at times when the chlorinator was operational, only a small percentage of the outflow received chlorination treatment, bypassing the chlorinator. After chlorination, the treated sewage flowed several hundred meters into Twelve Mile Creek. The depth of the water at the sample station ranged from one-half to two meters and all samples were collected from the surface.

5 - TWENTY MILE CREEK

The Twenty Mile Creek sample station (E) was located two miles west of Victoria Avenue on County Road 69 below a bridge spanning the creek (Plate VIII). At this location, the creek was approximately ten meters wide at normal water levels and ranged from one-half to one meter in depth. Livestock pastures and feedlots, including several poultry farms, were located upstream from the station. The poultry farms were interesting as poultry is a noted resevoir of Salmonella (Miner et al., 1967). All samples were collected from the water surface because the creek was shallow. During the winter months (January to March, 1972) the creek froze to such a depth that sampling was nearly impossible. Therefore, sampling was discontinued for this period.

PLATE VII - ST. CATHARINES POLLUTION CONTROL PLANT SEWAGE EFFLUENT SAMPLE STATION.
(NOTE THE CHLORINATION CHAMBER IN THE LOWER LEFT-HAND SIDE OF THE PLATE.)

PLATE VIII - TWENTY MILE CREEK SAMPLE STATION.

6 - SAMPLING METHOD

Pre-sterilized, galvanized buckets were used for collecting water samples at stations A, B, C and D. Samples were then poured directly into sterile, wide mouth, polypropylene jars which contained one ml per jar of a ten percent sodium thiosulfate solution added to neutralize any residual chlorine present in the water (STANDARD METHODS, 13th ed.). Specimens collected at sample station E, Twenty Mile Creek, were taken directly into sample bottles. During collection, the wide mouth end was held upstream to avoid accidental contamination from outside of the bottle.

Grab sampling was chosen for technical reasons and for convenience, although many studies incorporate the Moore Type swab for pathogen concentration (Grunnet and Nielsen, 1969; Claudon et al., 1971). The Moore swab usually consists of a gauze pad which is suspended in water for a period of time, thereby filtering a large unknown volume of water. However, by using grab sampling, a known volume of water could be filtered to concentrate pathogens so that quantitative methods could be used to enumerate the sewage indicator organisms and pathogens. In addition, for this study it would have been difficult to place and maintain Moore swabs at the beach and Twelve Mile Creek stations.

C - LABORATORY PROCEDURES

1 - PHYSICAL DETERMINATIONS

A Sargent Welch (model-S30009) pH meter was used for on-site determination of pH, and a Delta Scientific (model-85) temperature compensated oxygen-meter was similarly used for on-site measurements of dissolved oxygen. Nitrate N was determined as quickly as possible in the laboratory with a Hach (model-NI-10) nitrite-nitrate testing kit. After experiencing

some problems with the quality of reagents supplied with the kit during initial calibration procedures, all reagents were standardized according to the Brucine Method (STANDARD METHODS, 13th ed.). Ammonium N was calculated by the direct Nesslerization method (STANDARD METHODS, 13th ed.) with reagents and colorimeter supplied in the Hach kit (model-NI-8). Prior to testing, all samples were allowed to warm to 20C for maximum color development. Total inorganic phosphate (meta and ortho) P were determined by the Hach testing kit, model PO-23/PO-23A. Dissolved solids were determined by drying 100 ml of the sample at 103C for eight hours (constant weight) in chemically clean glassware after 100 ml samples were filtered through Millipore membrane filters of 0.45μ porosity (STANDARD METHODS, 13th ed.).

2 - BACTERIOLOGY

a - COLIFORM COUNTS

Prior to filtration, all water samples were well shaken and then diluted into glass bottles containing 99 ml of sterile phosphate buffer (pH 7.0). Aliquots of 100 ml were filtered using a sterile Millipore filter apparatus (XX1004700) onto sterile Millipore membrane filters of 47 mm diameter with a pore size of 0.45μ (STANDARD METHODS, 13th ed.). The filter disks were then placed onto suitable culture media (Plate IX). All media used were purchased either from Difco or BBL and are listed in Appendix I.

Filter disk duplicates of each serial dilution were placed on M-FC medium plus agar for faecal coliforms and M-Endo-LES agar for total coliform determinations by the membrane filtration (MF) method (Plate IX). The M-FC medium plus agar was then incubated at 44.5$\pm$0.2C for 22-24 hours in a water bath. The M-Endo-LES agar was incubated at 35.0$\pm$0.5C for 22$\pm$2 hours

PLATE IX - MILLIPORE FILTER APPARATUS.

(STANDARD METHODS, 13th ed.). Confirmation of presumptive coliforms, atypical or doubtful colonies, was carried out by incubating an isolate in lactose broth for 48 hours followed by transfer of positives to brilliant green lactose bile broth. Production of gas in the broth within 48 hours at 35.0±0.5C, typical growth on EMB or MacConkey's agar and Gram stain characteristics were used for confirmation of coliform members. Confirmation of faecal coliforms was carried out using lactose broth cultures transferred to EC broth and incubated for 24 hours at 44.5±0.2C in a water bath and observed for production of gas. Filter disks having 20-80 typical total coliform colonies and 20-60 typical faecal coliform colonies were considered to be statistically significant (STANDARD METHODS, 13th ed.). The multiple-tube fermentation technique employed replicate serial dilutions of the sample water into lactose broth or lauryl tryptose broth and was observed for acid and gas production. Results of the examination of replicate tubes and dilutions were expressed in terms of the Most Probable Number (MPN) which was based on certain probability formulas (STANDARD METHODS, 13th ed.).

For purposes of identification of coliform members, the following definitions as presented in STANDARD METHODS (13th ed.) were adopted:

> Multiple-tube fermentation technique definition for the coliform group - The coliform group comprises all of the aerobic and facultative anaerobic, Gram-negative, nonspore-forming, rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35.0C.
>
> Membrane filter technique for the coliform group - All the aerobic and facultative anaerobic, nonspore-forming, rod-shaped bacteria which produce a golden green metallic sheen within 24 hours of incubation on an Endo-type medium containing lactose are considered members of the coliform group.
>
> Membrane filter technique for faecal coliform group - All organisms which produce a blue colored colony on M-FC medium in 24 hours at 44.5±0.2C.

Duplicate estimations were run using both multiple-tube fermentation and MF methods for coliform estimations at the beginning of the study period. However, when results appeared to be comparative for both methods, the MPN method was dropped in favor of the less time consuming MF technique. Some well documented technical considerations have been outlined by Geldreich, Jeter and Winter (1967). Their recommendations on using accepted materials and procedures (STANDARD METHODS, 13th ed.) were followed closely. Care was taken to insure that size of sample, medium preparation, time and temperature for incubation and colony sheen discernment were proper for the MF technique. Confirmation and completed test procedures were carried out routinely on coliform colonies isolated on membrane filters to insure consistancy of enumeration techniques. Some technical limitations have been recognized in applying the MF method to chlorinated effluent (Geldreich, Jeter and Winter, 1967; STANDARD METHODS, 13th ed.; Bissonnette, Jezeski, McFeters and Stuart, 1974). These limitations have been considered in this study. Coliform data from the sewage effluent was calculated from MF counts in order to retain consistancy of methods between sample stations. However, chlorinated sewage effluent data was not applied in correlation and regression analysis. For the most part, the effluent had been chlorinated somewhat irregularly over three months.

b - SALMONELLA ISOLATIONS

Approximately two liters of each sample were filtered and concentrated by 0.45μ porosity membrane filtration (Millipore). When filtering water high in suspended matter, it became necessary to use a microfiber prefilter (type 25; Millipore Corp.). The prefilter was placed over a membrane filter and approximately 2-3 g of sterilized diatomaceous earth (J. T. Baker Co., 1939) were added aseptically over the prefilter.

After filtration, the diatomaceous earth, prefilter and membrane filter were aseptically divided and placed into single strength Selenite Cysteine broth and Tetrathionate broth to which a 1:1000 aqueous solution of Brilliant Green Dye was added. Both flasks were then gently shaken and incubated at 41.5±0.5C for 22±1 hours, plated, reincubated for an additional 24 hours and finally replated as recommended by Spino (1966).

Initially, both primary and secondary enrichment procedures using Tetrathionate broth and secondary plating at 24 and 48 hours were adopted. Later, however, secondary enrichment and plating at 48 hours were eliminated. It was found that primary selective enrichment recovered Salmonella in all instances and Tetrathionate broth was positive when viable Salmonella were present at 24 hours. This made additional incubation unnecessary. These findings agree with those of Spino (1966), Grunnet and Nielsen (1969) and Cherry et al. (1972).

After selective enrichment, Tetrathionate broth and Selenite broth cultures were streaked onto SS agar, Brilliant Green Sulfa agar, XLD agar, Hektoen agar and MacConkey's agar. The media were then incubated at 35.0± 1.0C for 18 to 24 hours. Typical Salmonella colonies were then identified by biochemical activity and serological reactions utilizing Salmonella poly H flagellar-antigen prepared by the Ontario Ministry of Health, Enteric Reference Laboratory, Toronto. Table 1 lists the biochemical tests which were performed and compared to identification procedures given by Edwards and Ewing (1972). All presumptive positives were submitted to the Ontario Ministry of Health, Enteric Reference Laboratory, Toronto for confirmation and serotyping.

TABLE 1 - PRELIMINARY IDENTIFICATION TESTS FOR *SALMONELLA*.[1]

Test	Expected Reaction
Hydrogen Sulfide[2]	+99% positive
Indole	—
Urease	—
Lysine	+
Citrate (Simmon's)	+98% positive
Ornithine	+
Malonate	—
Xylose	+
Arabinose	+
ONPG	—
Motility	+
Dextrose (Durham tube)	+

1 - Edwards and Ewing (1972)

2 - TSI agar

\+ - 90% or more positive within 1 or two days

— - 90% or more no reaction

D - ANALYSIS OF VARIANCE (ANOVA) BETWEEN SAMPLE STATIONS

Three replicate samples were taken from each station (Lake Ontario and Twelve Mile Creek on May 23, 1972, and sewage inflow and effluent and Twenty Mile Creek on June 1, 1972). Subsequently, three replicate counts were made from each sample. Count data was not transformed as the assumption of normality is usually not required for estimating the components of variance for most biological data (Steel and Torrie, 1960). Analysis of variance was carried out to establish that the primary sampling technique and subsequent enumeration methods (Steel and Torrie, 1960) produced comparable results in duplicate samples. Results of the analysis indicated that the variation in counts from different primary samples was not significant (Summary Table 2) except for total coliform replicates from the sewage effluent station. This probably indicated clustered occurrence of bacteria (one of the hazards of the sampling technique). Results of analysis of variance within samples indicated that the enumeration techniques were adequate.

One particular problem, inherent in the membrane filtration technique for coliforms, was occasionally encountered in counting coliform colonies on Endo-LES agar. This problem occurs when water samples contain large numbers of Gram-negative bacteria (other than coliforms). These tend to inhibit normal pigment development of the coliform colonies. Raw sewage, with its large bacterial flora, falls into this category. Therefore, confirmation procedures had to be used occasionally in sewage analysis. Infrequently, counts had to be determined from membrane filters having less than the desired number of colonies for statistical significance (STANDARD METHODS, 13th ed.).

TABLE 2 - ANALYSIS OF VARIANCE (ANOVA) RESULTS FROM EACH SAMPLE STATION COMPARING VARIATION IN THE THREE ENUMERATIONS WITHIN PRIMARY SAMPLES TO VARIATION BETWEEN THE THREE PRIMARY SAMPLES.

	Total Coliform	Faecal Coliform
Lake Ontario	F = 0.98 not significant	_____*
Twelve Mile Creek	F = 0.21 not significant	F = 0.17 not significant
Sewage Inflow	F = 0.12 not significant	F = 0.91 not significant
Sewage Effluent	F = 1615.7 significant P = 0.005	_____*
Twenty Mile Creek	F = 2.47 not significant	F = 0.01 not significant

* - no faecal coliform organisms isolated

P = 0.05 F significant at 5.14

III RESULTS

A - COLIFORM LEVELS

Some interesting differences in coliform levels between the sample areas were noted early in the sampling program. Figure 2 shows seasonal variation of total and faecal coliform levels in the sewage inflow. Population levels of total and faecal organisms remained relatively constant over the sample period except for minor fluctuations. Faecal coliforms appeared to comprise roughly 5-15% of total coliform levels on many sample dates. These results are equivalent to the results of Gallagher and Spino (1968) who noted that faecal coliforms comprised approximately 15% of total coliforms from many river systems throughout the United States. Levels of total and faecal coliforms in the sewage effluent (Figure 3) appeared to be somewhat more erratic than the populations in the sewage inflow. In addition to their more erratic fluctuations, both total and faecal coliform levels were lower in the effluent than the sewage inflow. The most dramatic differences between the behavior of inflow and effluent populations were produced by chlorination of the effluent from mid-May to the end of September. The faecal coliforms seemed to have been eliminated faster than the non-faecal component of the total coliforms by the chlorination process. Occasional shutdowns of the chlorination process added greater inconsistancies to observed coliform levels during the summer period by causing population peaks when the chlorinator was inoperable. In general, faecal coliform levels comprised a smaller proportion of the total coliform population in the sewage effluent than in the inflow. For example, on September 30, 1971, faecal organisms comprised 10% of the total numbers in

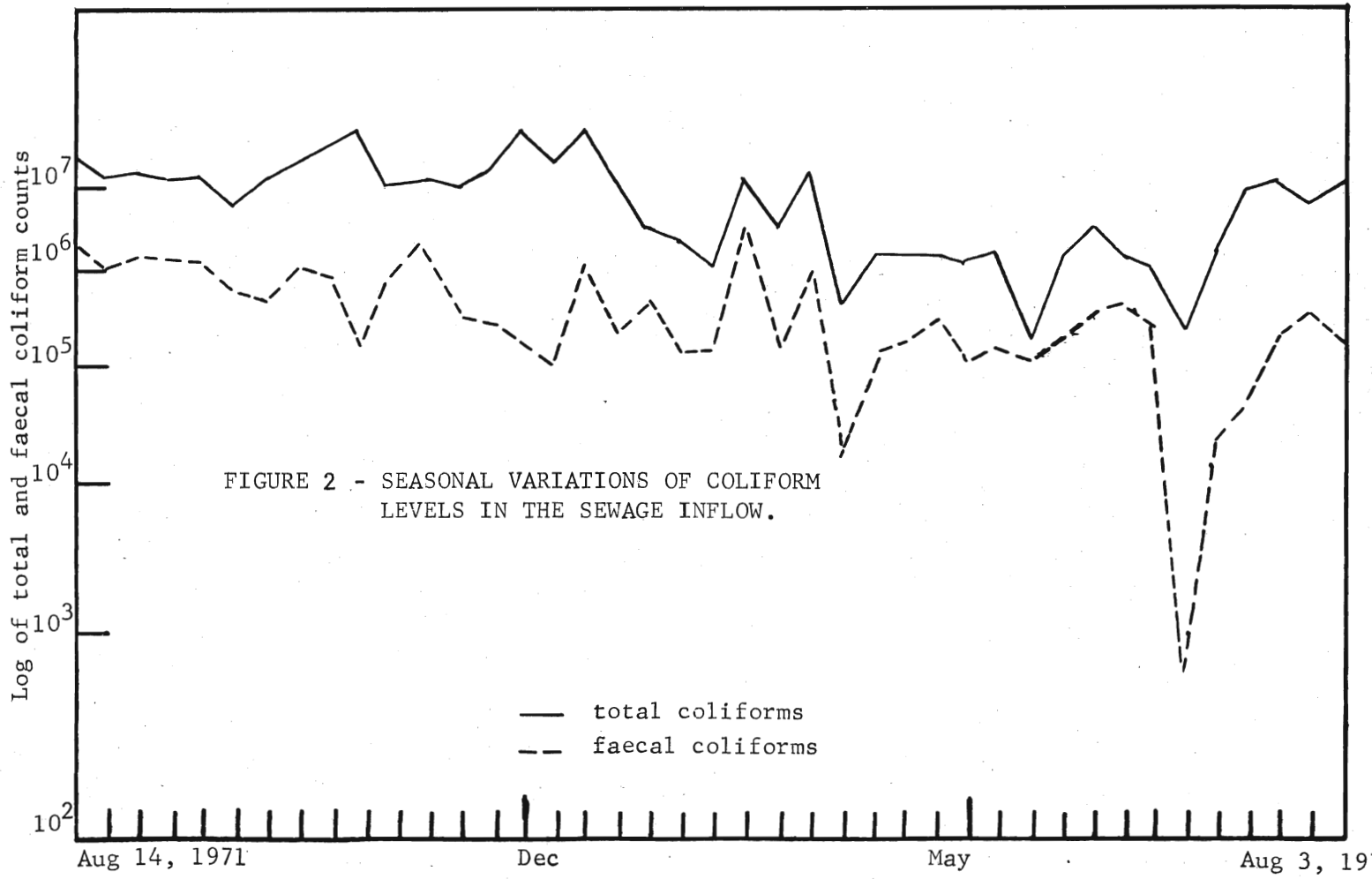


FIGURE 2 - SEASONAL VARIATIONS OF COLIFORM LEVELS IN THE SEWAGE INFLOW.

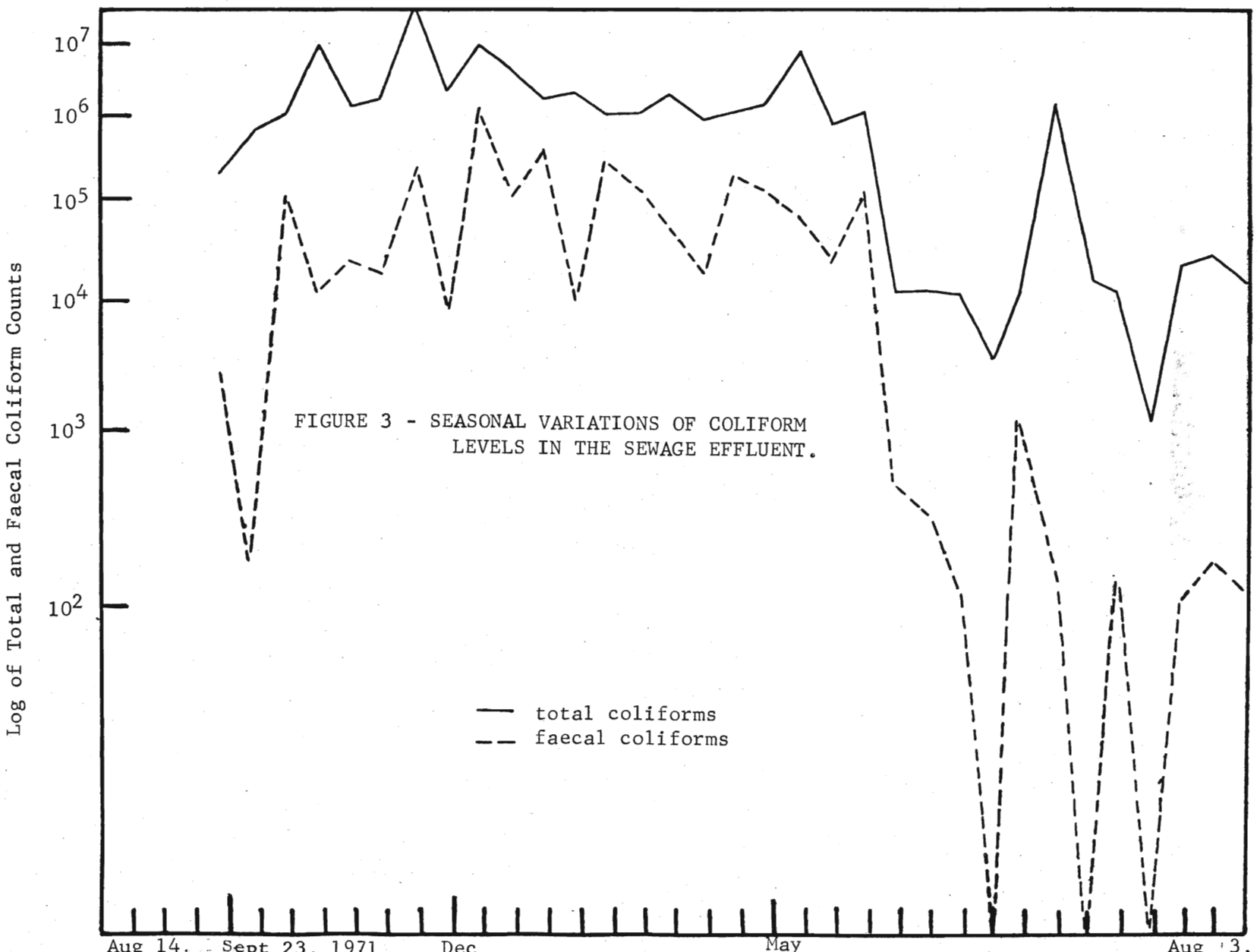


FIGURE 3 - SEASONAL VARIATIONS OF COLIFORM LEVELS IN THE SEWAGE EFFLUENT.

the inflow compared to 0.03% in the effluent, on June 28, 1972, faecal coliforms comprised 0.2% in the inflow and 0.02% in the effluent and on July 6, 1972, they comprised 2% in the inflow compared to $<$0.003% in the effluent (raw data in Appendix II). When the chlorinator was operational, the effects of the treatment was obvious. This was evident on June 13, 1972, July 6, 1972 and July 14, 1972 (Figure 3) when faecal organisms were reduced to levels approaching zero.

Total and faecal coliform levels in Twelve Mile Creek (Figure 4) showed extreme fluctuations throughout the sampling period. For the most part, both total and faecal coliform population levels appeared to be notably less than those of the sewage effluent, except when the effluent was effectively chlorinated. Population minima for faecal coliforms in Twelve Mile Creek were observed on March 28, June 28 and July 14, 1972. There was considerable variation in the percentage of faecal organisms comprising the total coliform group in Twelve Mile Creek. At times, faecal coliforms made up a large percentage. For example, on December 22, 1971, the faecal coliform population was approximately 63% of the total, and on July 25, 1972, it was approximately 57%. At other times the faecal organisms made up a very small percentage, as seen on March 25, 1972, when they made up 0.3% of the total, and on July 14, 1972, when total coliform numbers were at 1.4×10^4, while faecal levels were not detected.

Total coliform levels in Lake Ontario (Figure 5) remained relatively constant over the sample period except for a decline in the fall of 1971 and the summer of 1972. However, faecal coliform levels were observed to rise and fall in an erratic pattern with a marked decline for the summer.

FIGURE 4 - SEASONAL VARIATIONS OF COLIFORM LEVELS IN TWELVE MILE CREEK.

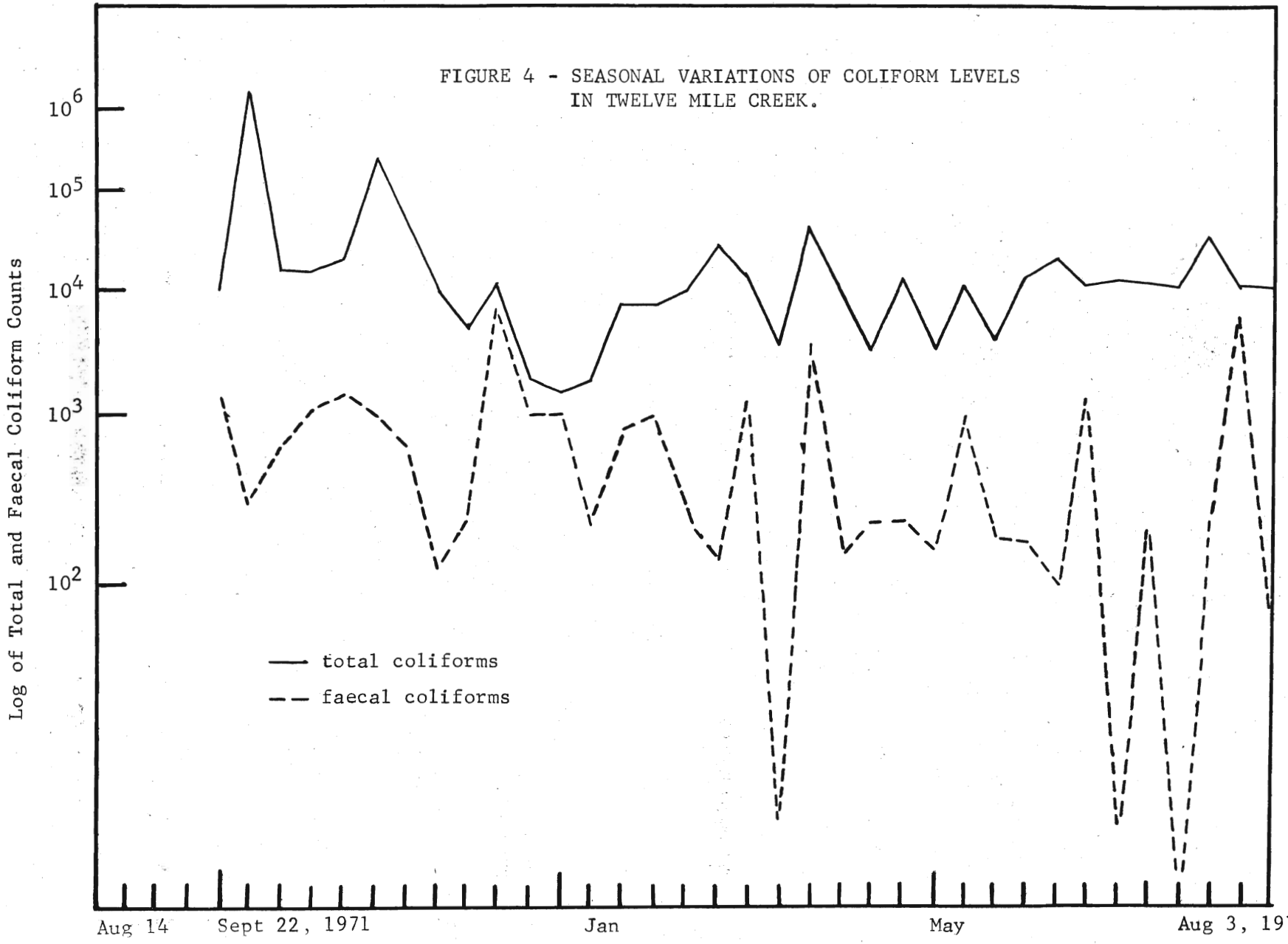
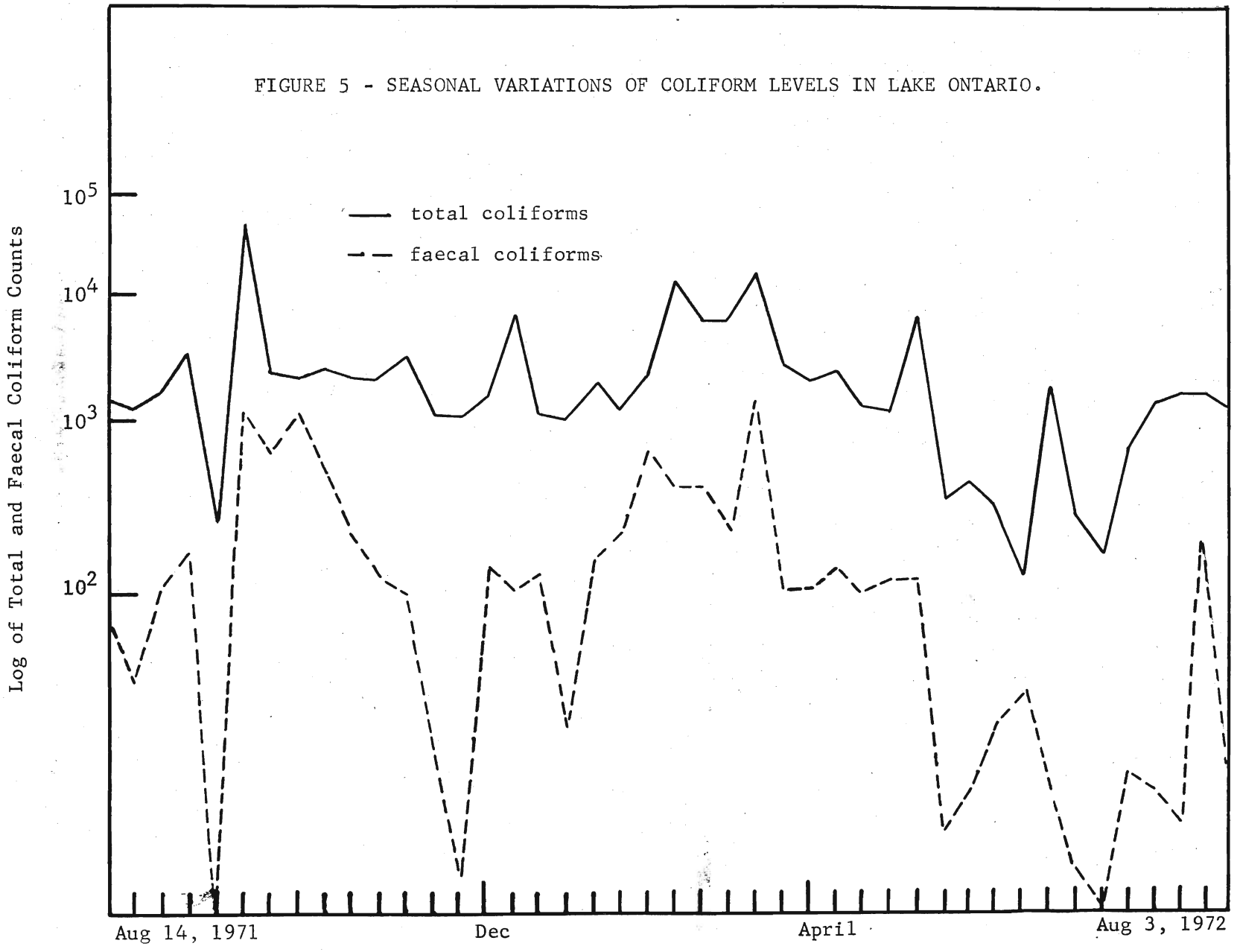


FIGURE 5 - SEASONAL VARIATIONS OF COLIFORM LEVELS IN LAKE ONTARIO.



Generally, faecal coliform levels made up only a small portion of the total coliform population and on two sample dates only non-faecal coliforms were isolated. These were September 23, 1971 and June 28, 1972.

Considering the overall population levels of total and faecal coliforms present in the sewage inflow, sewage effluent, Twelve Mile Creek and Lake Ontario, decreasing population levels prevailed between each sample station. That these sample stations had populations which differed in size can be seen from the geometric mean (Table 3). The geometric means were calculated in order to minimize the effects of a few extreme measurements (Guidelines for Water Quality Management; and Carruthers, no date). Based on the geometric means, total coliform levels of the sewage effluent were only 5% of the inflow levels, Twelve Mile Creek of the effluent 5% and Lake Ontario of Twelve Mile Creek 13%. Similarly, the faecal levels in the succeeding sample stations, compared to the previous ones, were 1%, 7% and 25% respectively. The significance of these observed differences in population levels was demonstrated comparing arithmetic means (Tables 4 and 5) by analysis of variance (ANOVA) and t test (Student's t test using pooled variance; Steele and Torrie, 1960). Whereas the variation between populations and between means of adjacent sample stations were always significant in the case of faecal coliforms, two exceptions were noted in the case of the total coliforms. The differences between the sewage inflow and effluent and Twelve Mile Creek and Lake Ontario were not significant, both for variation between populations and for differences between their means. Whereas there was a decrease in the percentage composition of faecal coliforms of the total population from the sewage inflow to the effluent ($5.0\pm1.2\%$-$1.4\pm1.6\%$),

TABLE 3 - SUMMARY OF DATA FROM ALL SAMPLE STATIONS INCLUDING GEOMETRIC MEAN AND RANGES OF COLIFORM COUNTS.

	Sewage Inflow	Sewage Effluent	Twelve Mile Creek	Lake Ontario	Twenty Mile Creek
Total Coliform*	4.0×10^5- 8.0×10^7	1.3×10^3- 6.5×10^7	2.6×10^3- 3.0×10^5	1.5×10^2- 7.0×10^4	1.0×10^2- 1.8×10^4
Geometric Mean	8.6×10^6	4.3×10^5	1.9×10^4	2.4×10^3	9.0×10^2
Faecal Coliform*	8.0×10^2- 5.8×10^6	0- 1.8×10^6	0- 1.0×10^4	0- 1.4×10^3	0- 8.0×10^2
Geometric Mean	4.3×10^5	6.2×10^3	4.4×10^2	1.1×10^2	1.3×10^2
Temp ^oC	10.5- 23.0	11.0- 23.0	2.0- 21.5	2.0- 20.0	2.0- 24.5
pH	5.8- 8.3	7.2- 8.0	7.7- 8.3	7.0- 8.4	7.4- 8.1
DO ∓	2.5- 6.5	0.9- 8.5	4.0- 10.2	3.5- 10.2	3.0- 9.2
PO_4-P ∓	0.67- 2.67	0.07- 2.67	0.07- 0.33	0.07- 0.33	0.07- 0.27
NO_3-N ∓	0.2- 4.0	0.2- 4.0	0.13- 0.40	0.12- 0.42	0.12- 0.62
NH_4-N ∓	3.0- 22.0	3.0- 24.0	0.2- 0.6	0.2- 0.4	0.2- 0.8
TDS ∓	220- 1300	200- 1300	200- 1500	200- 600	300- 1200

* - bacteria per 100 ml

∓ - ppm

DO - dissolved oxygen

TDS - total dissolved solids

TABLE 4 - ANALYSIS OF VARIANCE FOR TOTAL COLIFORM ESTIMATIONS BETWEEN ADJACENT SAMPLE STATIONS AND t TEST FOR DIFFERENCES BETWEEN TWO MEANS USING POOLED VARIANCE.

	Twelve Mile Creek	sewage effluent	sewage inflow
Lake Ontario	$F = 1.79$ ∓ not significant $S_d = \sqrt{\frac{2S^2}{r}} = 3.26 \times 10^3$ $\bar{X}_1 - \bar{X}_2 = 1.14 \times 10^4$ $lsd_{(0.05)} = 6.53 \times 10^3$		
Twelve Mile Creek		$F = 4.64$ ∓ significant at 0.05 $S_d = \sqrt{\frac{2S^2}{r}} = 2.0 \times 10^5$ $\bar{X}_1 - \bar{X}_2 = 4.18 \times 10^5$* $lsd_{(0.05)} = 4.0 \times 10^5$	
sewage effluent			$F = 2.2$ ∓ not significant $S_d = \sqrt{\frac{2S^2}{r}} = 4.36 \times 10^5$ $\bar{X}_1 - \bar{X}_2 = 1.67 \times 10^6$ $lsd_{(0.05)} = 2.14 \times 10^4$

∓ - df = 64, F sig. at 4.0 when P = 0.05

* - significant

TABLE 5 - ANALYSIS OF VARIANCE FOR FAECAL COLIFORM ESTIMATIONS BETWEEN ADJACENT SAMPLE STATIONS AND t TEST FOR DIFFERENCES BETWEEN TWO MEANS USING POOLED VARIANCE.

	Twelve Mile Creek	sewage effluent	sewage inflow
Lake Ontario	$F = 8.11$ ‡ significant at 0.01 $S_d = \sqrt{\frac{2S^2}{r}} = 28$ $\bar{X}_1 - \bar{X}_2 = 80$* $lsd_{(0.05)} = 56$		
Twelve Mile Creek		$F = 6.0$ ‡ significant at 0.025 $S_d = \sqrt{\frac{2S^2}{r}} = 8.1 \times 10^3$ $\bar{X}_1 - \bar{X}_2 = 2.0 \times 10^4$* $lsd_{(0.05)} = 1.62 \times 10^4$	
sewage effluent			$F = 9.5$ ‡ significant at 0.005 $S_d = \sqrt{\frac{2S^2}{r}} = 1.88 \times 10^4$ $\bar{X}_1 - \bar{X}_2 = 5.8 \times 10^4$* $lsd_{(0.05)} = 3.8 \times 10^4$

‡ - df = 64, F sig. at 4.0 when P = 0.05

* - significant

the reverse was true between Twelve Mile Creek and Lake Ontario (2.3±1.4%-5.4±1.2%). The faecal coliform composition of the sewage effluent and Twelve Mile Creek was nearly equal with 1.4±1.6%-2.3±1.4%, respectively.

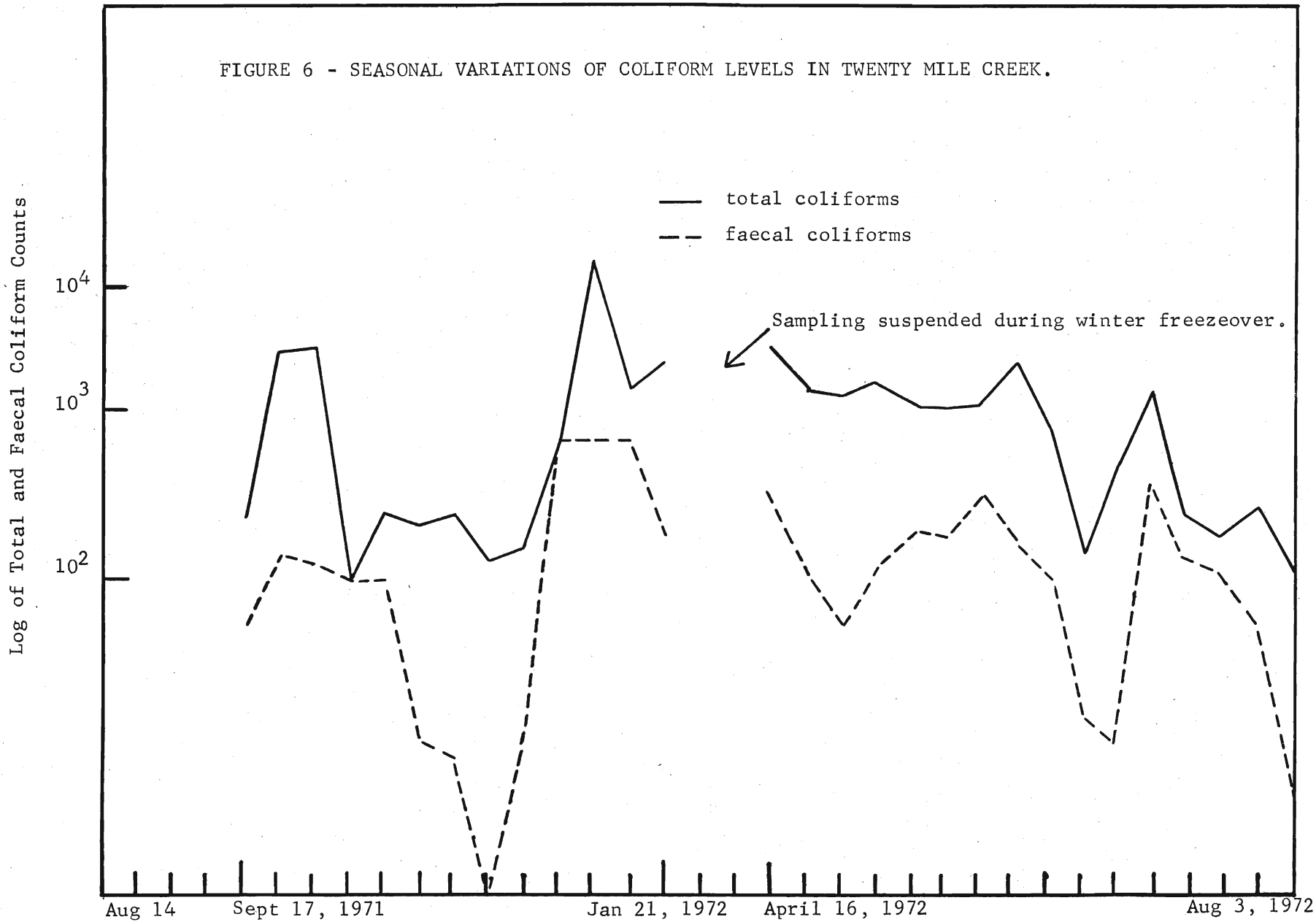
Twenty Mile Creek (Figure 6) total and faecal coliform population levels were very erratic over most of the sample period. On several sample days, for example November 3, 1971 and April 21, 1972, faecal coliform levels decreased notably, while the total coliform population remained relatively stable. By contrast, on October 4, 1971 and December 10, 1971, the faecal population also made up a large percentage of the total coliform bacterial level. Based on the geometric means (Table 3), faecal coliforms comprised 14.4% of the total population in Twenty Mile Creek. This percentage was very similar to the previously mentioned results of Gallagher and Spino (1968).

B - PHYSICAL AND CHEMICAL FACTORS

The physical parameters, temperature, pH and dissolved oxygen, and the chemical parameters, total inorganic phosphate P, ammonium N and nitrate N (Appendix II), were monitored at the same time as bacterial populations in an attempt to find out if differences in population levels at the various sample sites could be correlated with differences in these parameters.

The most obvious physical or chemical factor which showed both seasonal and between sample station variation was temperature. The temperature ranges for Lake Ontario, Twelve Mile Creek and Twenty Mile Creek were very similar. Twelve Mile Creek and Twenty Mile Creek appeared to be somewhat more sensitive to ambient temperature changes. However, Twenty Mile Creek did warm to 24.5C as the waterflow all but stopped during the dry summer months. Lake Ontario normally was least subject to abrupt temperature

FIGURE 6 - SEASONAL VARIATIONS OF COLIFORM LEVELS IN TWENTY MILE CREEK.



fluctuations, although, a rapid decrease of water temperature was registered on several occasions. For example, during a violent storm on July 28, 1972, an upwelling of cold water lowered the surface temperature of the lake from 16.0 to 13.0C. Water temperature for the sewage inflow and effluent during the coldest months only lowered to approximately 11.0C, from c. 22.0C common during the warm months, due to the "closed system" protection of the sewage treatment process from the elements. The pH ranges for Lake Ontario, Twelve Mile Creek, Twenty Mile Creek and sewage effluent were similar. However, the sewage inflow was subject to rapid changes which might have been produced by alkaline or acid industrial wastes. Few notable pH changes occurred at the other sample stations.

Nutrient levels appeared to be directly related to amounts of sewage contamination present in the water at the sample stations. In addition, Twenty Mile Creek drained a largely agricultural watershed and likely contained varying amounts of leached fertilizer nitrates and suspended phosphates carried in particulate matter. The sewage inflow and effluent regularly contained high levels of nutrients. Nutrient levels in the sewage inflow were lowest during August and September of 1971, after which levels increased, then reached a plateau and varied randomly thereafter. However, a slight decrease in ammonium nitrogen was noted during May and June, 1972, which might have been the result of dilution by large amounts of rainfall during those months. Sewage effluent nutrient level variations expectedly followed the same pattern as the sewage inflow, however, ammonium nitrogen levels were regularly higher in the effluent, possibly as a result of bacterial degradation of nitrogen compounds. Increases of nutrient levels were noted for both sample stations during the December to May period when

nutrient removal systems were less efficient due to unfavorable cold temperatures. Nutrient levels in Twenty Mile Creek appeared to vary randomly except for a slight decrease of ammonium nitrogen levels noted during the summer months. Total dissolved solid ranges were similar for most sample stations, the exception being Lake Ontario which normally contained lower amounts. No seasonal variations were observed. However, slightly increased levels were noted during periods of rainfall on several occasions.

C - CORRELATION AND REGRESSION ANALYSIS

Statistical analysis was applied to the results in order to determine if any linear relationship existed between the dependent variables (the coliform populations) and the independent variables (the environmental and nutritional factors). If a relationship did exist, it was also necessary to determine the degree of relatedness. Correlation and regression analysis were carried out to answer the above questions. By applying regression analysis, a prediction could be made on the variation of the dependent variable from the variation of the independent variable. However, this would not necessarily infer a causal relationship. It was possible that bacterial population fluctuations observed in this study were the result of more complex interactions. Linear regression program LIST 80, B grow 2 analysis was carried out on a Burroughs 5500 computer at Brock University, St. Catharines, Ontario.

Only linear regression analysis was carried out in a search for a relationship between the coliform populations and the independent variables. Other studies by Coleman et al.(1974), Brasfield (1972) and Cassie (1961) have detected a relation with similar population data by linear regression analysis. Correlation results between total and faecal coliform count

TABLE 6 - BETTER CORRELATIONS BETWEEN TOTAL AND FAECAL COLIFORMS AND THE INDEPENDENT VARIABLES WITH THE LINEAR REGRESSION EQUATION FOR THOSE BETTER CORRELATIONS.

Station	O_2	pH	TDS	Temperature	NO_3	PO_4	NH_3
Lake Ontario TC	—	—	—	—	—	—	—
Lake Ontario FC	—	—	—	—	r = 0.26 Y = 1200 + -34X	r = 0.27 Y = 1100 + 79X	—
Twelve Mile Creek TC	—	—	—	—	—	—	—
Twelve Mile Creek FC	—	—	—	—	—	—	—
Sewage Inflow TC	—	—	—	—	—	—	—
Sewage Inflow FC	—	—	—	—	r = -0.51 Y = -88 + 230X	—	—
Sewage Effluent TC	—	—	—	—	—	r = 0.37 Y = 1400 + 930X	—
Sewage Effluent FC	—	—	—	—	—	r = 0.48 Y = 490 + 390X	—
Twenty Mile Creek TC	—	—	—	—	—	r = 0.34 Y = 170 + 370X	r = 0.39 Y = 11 + 3.5X
Twenty Mile Creek FC	—	—	—	r = -0.42 Y = -15 + 470X	—	r = 0.33 Y = 1300 + 39X	r = 0.31 Y = 94 + 110X

r = linear correlation coefficient TC - total coliform FC - faecal coliform

data and the independent variables are shown for the better correlations in Table 6. Graphs showing the regression of coliform counts on nutrient levels and temperature of the better correlations appear in Appendix IV with the line of best fit plotted by computer.

D - SALMONELLA RESULTS

The overall percentage of Salmonella isolations relative to total isolation attempts from all sample stations (Table 7) was 18.5% from 178 asseys. Only slight differences between sample stations were noted. The sewage inflow produced the highest percentage of 22.9%, whereas Twenty Mile Creek produced the lowest at 15.4%. Ten different serotypes of Salmonella were identified from 32 isolations from all sample stations (Table 8) and include:

S. typhimurium	S. manhattan
S. newport	S. typhimurium var copenhagen
S. heidelberg	S. bareilly
S. montevideo	S. muenster
S. saint paul	S. kottbus

Of the ten serotypes isolated, no one serotype appeared to be limited to any one particular sample station.

It appeared that the major portion of isolations were distributed largely during the months of November through June inclusive with few isolations occurring in the summer and fall. Thus, further analysis was carried out. The numbers of Salmonella isolated from all sites pooled together were plotted for each month (Figure 7). It appeared that higher numbers of Salmonella were viable in the water during the winter and spring than at other times of the year. The bulk (93%) of isolations from Twelve Mile Creek and Lake Ontario (Figure 8) were distributed within the November

TABLE 7 - RELATIVE NUMBER OF SAMPLES POSITIVE FOR *SALMONELLA* FOR ALL SAMPLE POINTS.

Station	Total Number of Samples	*Salmonella* Positives	Percentage of Positives
Sewage Inflow	41	8	22.9%
Sewage Effluent	33	6	18.2%
Twelve Mile Creek	35	7	20.0%
Lake Ontario	43	7	16.3%
Twenty Mile Creek	26	4	15.4%
Totals	178	32	18.5%

TABLE 8 - SALMONELLA ISOLATIONS WITH TEMPERATURE AND COLIFORM COUNTS.

Station	Date	Water Temp °C	Coliforms Total	Coliforms Faecal	Salmonella Species
	Nov 4/71	20.5	2.3×10^{7}	4.0×10^{6}	newport
	Dec 29/71	13.0	2.6×10^{7}	4.0×10^{5}	heidelberg
Sewage	Feb 6/71	12.5	2.2×10^{7}	5.8×10^{6}	montevideo
	Mar 9/72	11.0	6.7×10^{5}	3.0×10^{4}	muenster
	May 4/72	13.5	2.0×10^{6}	1.4×10^{5}	muenster
Inflow	May 16/72	15.0	3.7×10^{5}	1.5×10^{5}	muenster
	Jun 13/72	17.5	2.3×10^{6}	6.4×10^{5}	typhimurium
	Jun 21/72	21.5	1.4×10^{6}	4.6×10^{5}	typhimurium
	Dec 29/71	14.5	7.0×10^{6}	1.0×10^{5}	typhimurium
Sewage	Feb 24/72	12.0	1.0×10^{6}	3.0×10^{4}	kottbus
	Mar 9/72	11.0	2.5×10^{6}	3.5×10^{5}	muenster
	Mar 28/72	11.0	3.3×10^{6}	1.9×10^{5}	muenster
Effluent	Apr 27/72	13.0	9.0×10^{5}	4.3×10^{4}	manhattan
	May 16/72	16.0	1.7×10^{4}	7.0×10^{2}	typhimurium
	Nov 4/71	15.4	6.9×10^{4}	8.0×10^{2}	newport
Twelve	Jan 27/72	4.0	9.0×10^{3}	9.0×10^{2}	typhimurium
	Feb 6/72	3.0	9.0×10^{3}	1.0×10^{3}	typhimurium
Mile	Feb 24/72	2.0	5.0×10^{4}	2.4×10^{2}	var copenhagen bareilly
	Mar 28/72	3.0	6.0×10^{3}	20	saint paul
Creek	May 4/72	6.0	5.4×10^{3}	4.0×10^{2}	montevideo
	May 16/72	8.0	5.7×10^{3}	2.6×10^{2}	typhimurium
	Aug 24/71	19.5	1.6×10^{3}	65	typhimurium
	Dec 1/71	10.0	2.8×10^{3}	2.0×10^{2}	newport
Lake	Dec 27/71	4.5	1.0×10^{3}	50	heidelberg
	Jan 27/72	2.0	4.0×10^{3}	8.0×10^{2}	bareilly
Ontario	Feb 24/72	3.0	7.9×10^{3}	3.9×10^{2}	kottbus
	May 12/72	8.0	8.0×10^{3}	1.6×10^{2}	kottbus
	May 16/72	10.0	5.4×10^{2}	20	muenster
Twenty	Sep 17/71	18.0	3.9×10^{2}	80	newport
	Nov 25/71	5.0	2.4×10^{2}	40	newport
Mile	Apr 6/72	6.0	5.1×10^{3}	5.2×10^{2}	muenster
Creek	Apr 21/72	11.0	1.8×10^{3}	80	montevideo

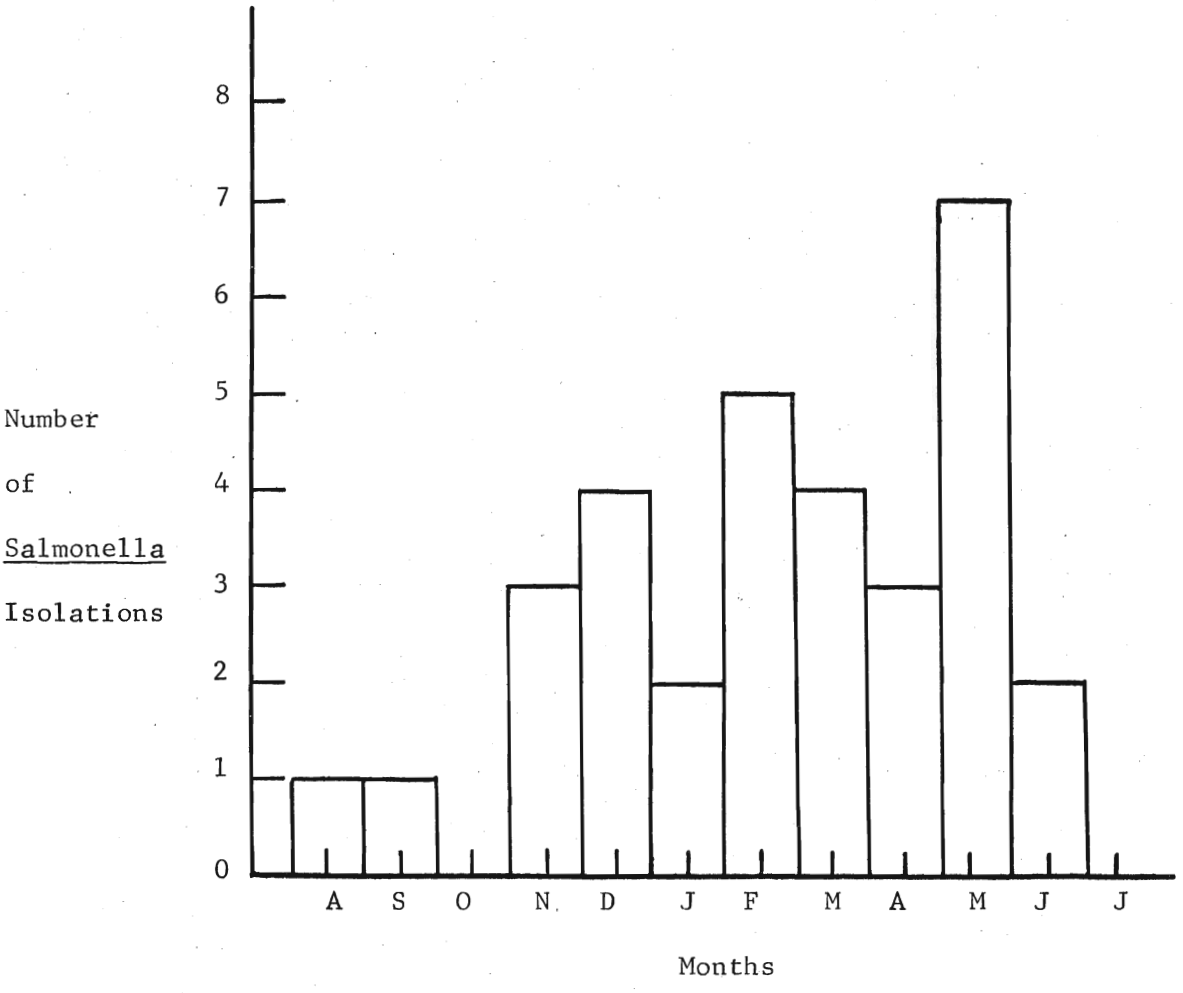


FIGURE 7 - MONTHLY DISTRIBUTION OF SALMONELLA ISOLATIONS FOR ALL SAMPLE STATIONS.

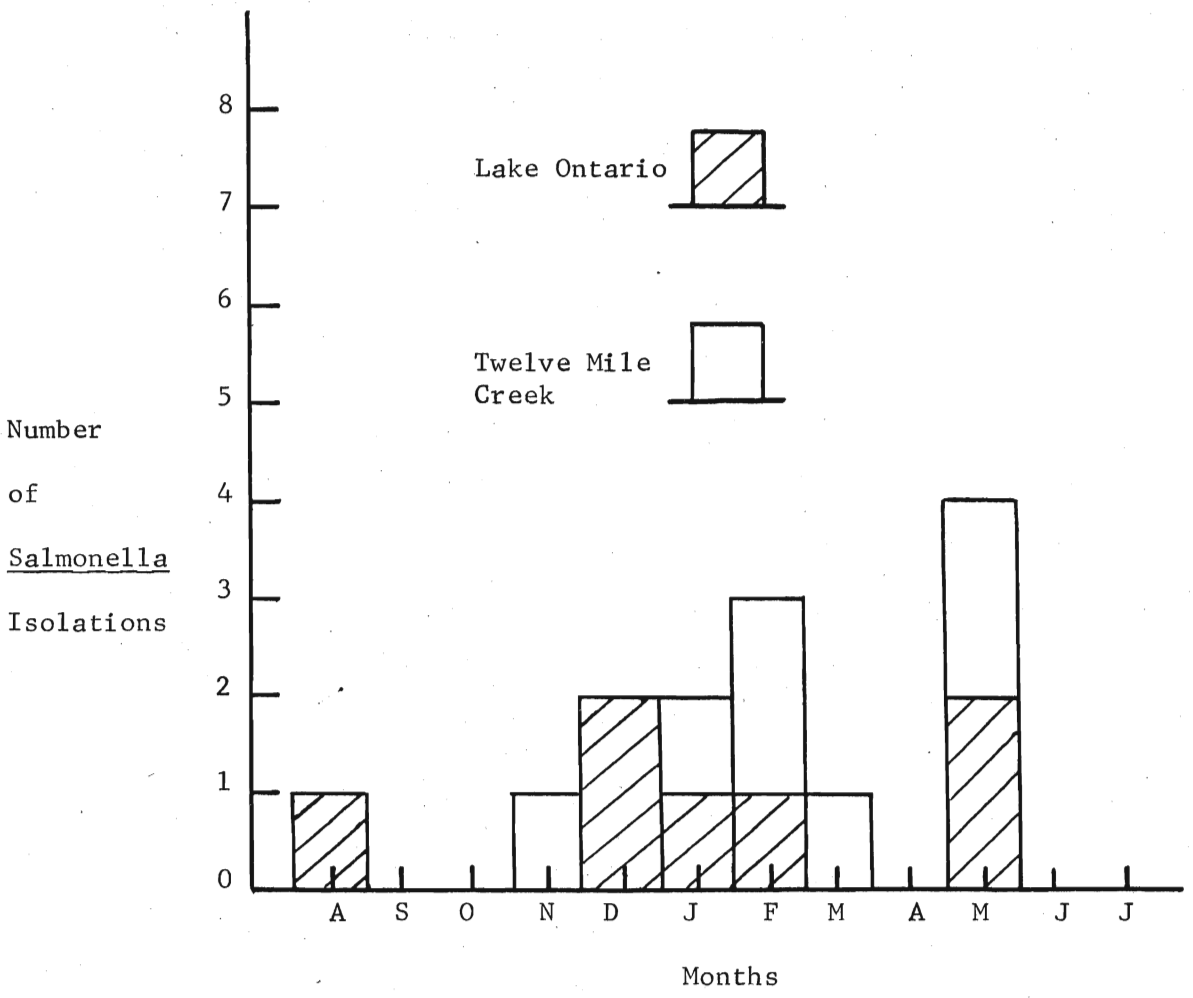


FIGURE 8 - MONTHLY DISTRIBUTION OF SALMONELLA ISOLATIONS FROM LAKE ONTARIO AND TWELVE MILE CREEK.

through May period. The results for the sewage effluent included chlorinated samples and may have been influenced by the lethal effect of the chlorination process. Approximately 84% of the Salmonella isolations from the sewage inflow and effluent (Figure 9) occurred during the November through May period. These results suggested the existance of a relationship between Salmonella isolations and temperature. When the Salmonella isolations for all sample stations were plotted (Figure 10) as a function of temperature, the greatest portion of the isolations appeared in the four coldest temperature ranges, 0-4.0, 4.1-8.0, 8.1-12.0 and 12.1-16.0C. These included the warmer-skewed sewage results. The sewage cooled only to 10.5C, and, therefore, the isolations which occurred in the two ranges, 8.1-12.0 and 12.1-16.0C, included all the cold temperature sewage results. It was quite possible that if the sewage had been further cooled, a greater number of Salmonella isolations would have been observed in the 0-10.0C range. However, there remained the possibility that the lower range distribution of positive Salmonella isolations was merely a function of fewer isolation attempts during warmer weather. The percentage of positive isolations was plotted by temperature class in order to determine whether positive Salmonella isolations were a function of number of isolation attempts. Taking both percentage of positive attempts and actual number of isolations from Lake Ontario and Twelve Mile Creek into account (Figure 11), it is clear that most isolations occurred when water temperatures were low. Only one isolation in the 12.0-16.0C range was noted out of two attempts for Twelve Mile Creek. This, of course, produced an abnormally tall bar in that range.

The percentage of isolations for the sewage inflow (Figure 12) appear to be evenly distributed over all temperature ranges with a slight peak in

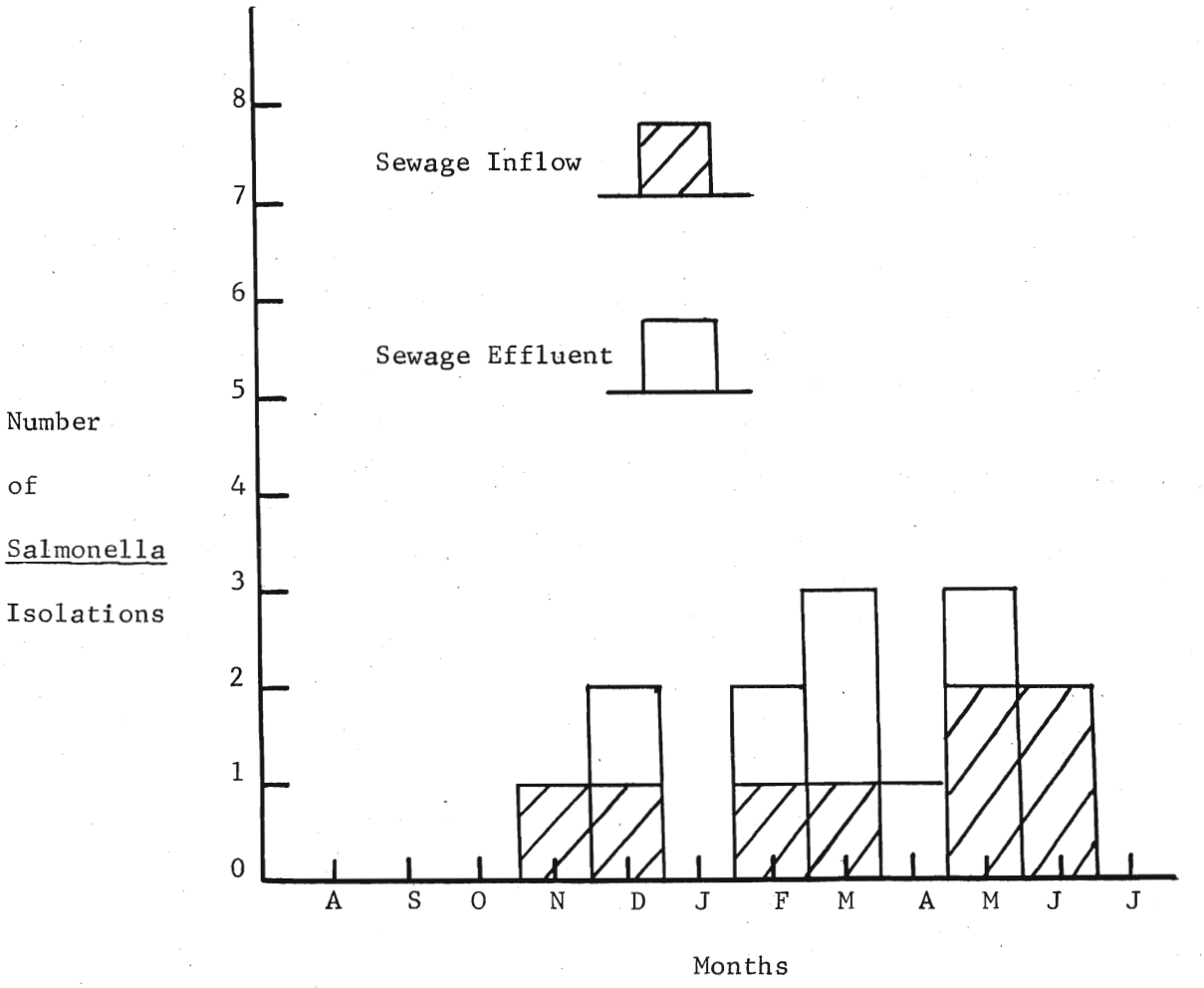


FIGURE 9 - MONTHLY DISTRIBUTION OF SALMONELLA ISOLATIONS FROM SEWAGE INFLOW AND EFFLUENT.

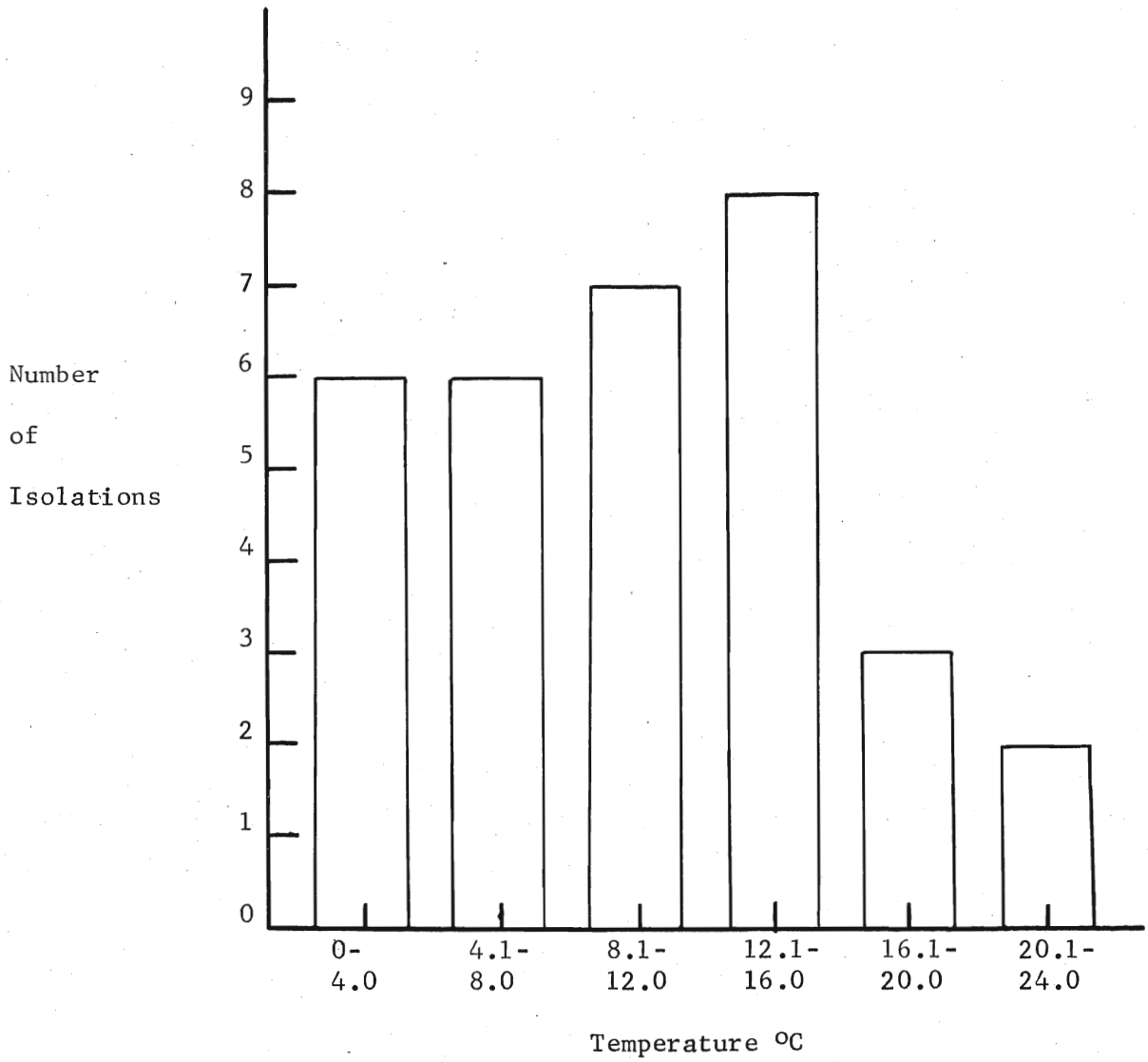
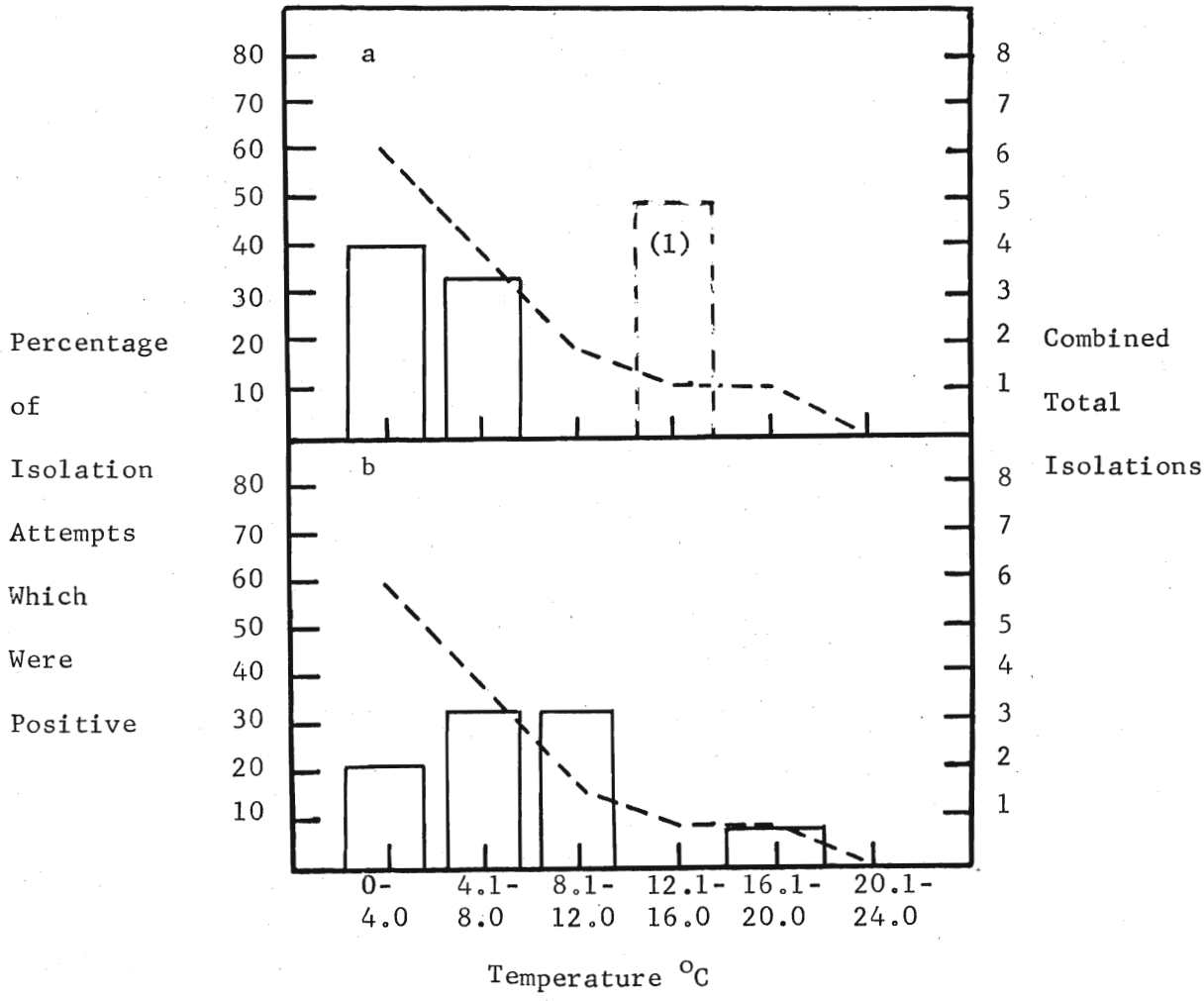
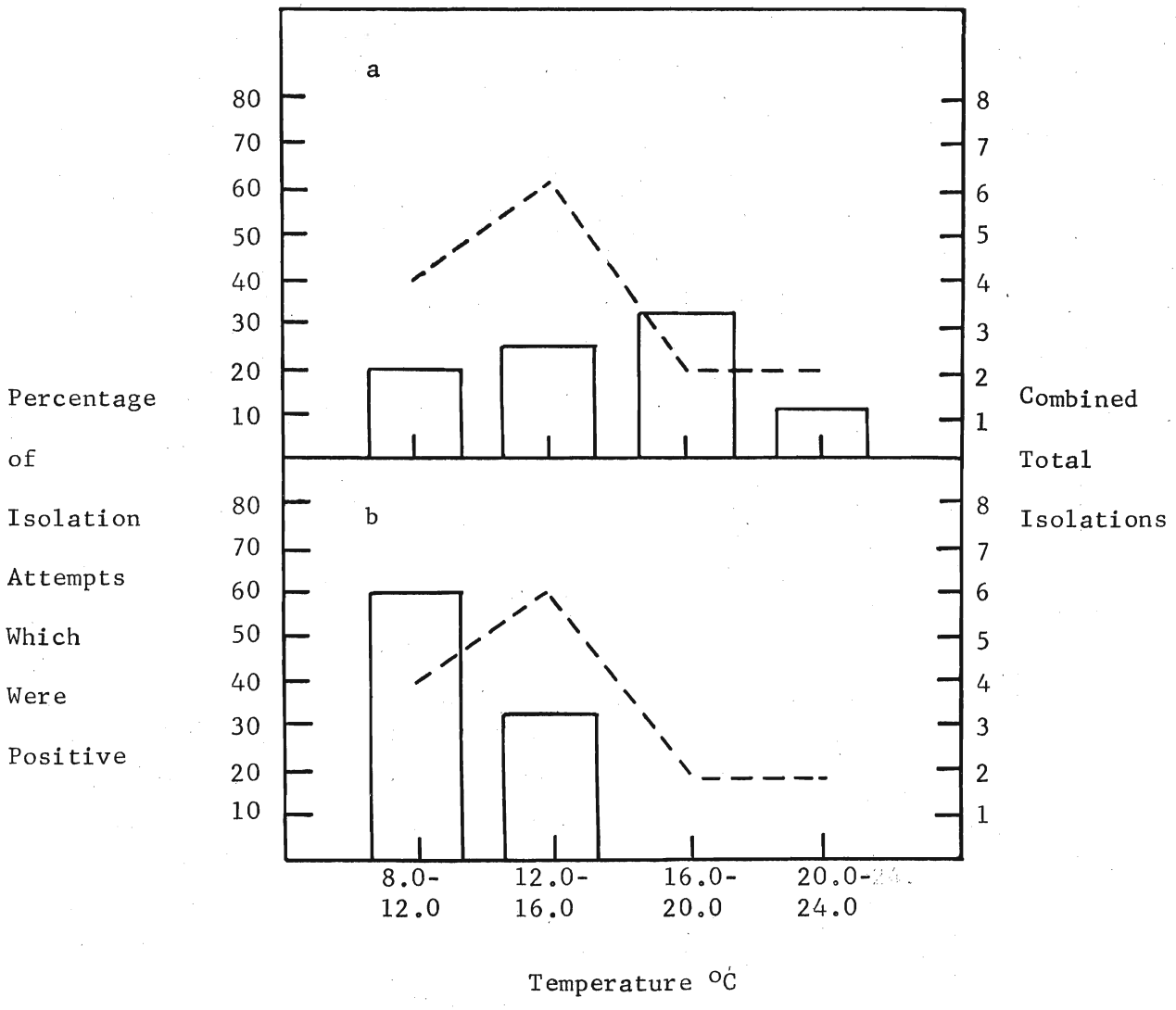


FIGURE 10 - RELATIONSHIP OF SALMONELLA ISOLATIONS FROM ALL SAMPLE STATIONS TO TEMPERATURE.



a - Twelve Mile Creek
b - Lake Ontario
(1) - only two attempted isolations fell within this temperature range
– – – combined total isolations for Twelve Mile Creek and Lake Ontario

FIGURE 11 - RELATIONSHIP OF SALMONELLA ISOLATIONS FROM LAKE ONTARIO AND TWELVE MILE CREEK TO TEMPERATURE.



a - Sewage inflow
b - Sewage effluent
— — — combined total isolations for the sewage inflow and effluent

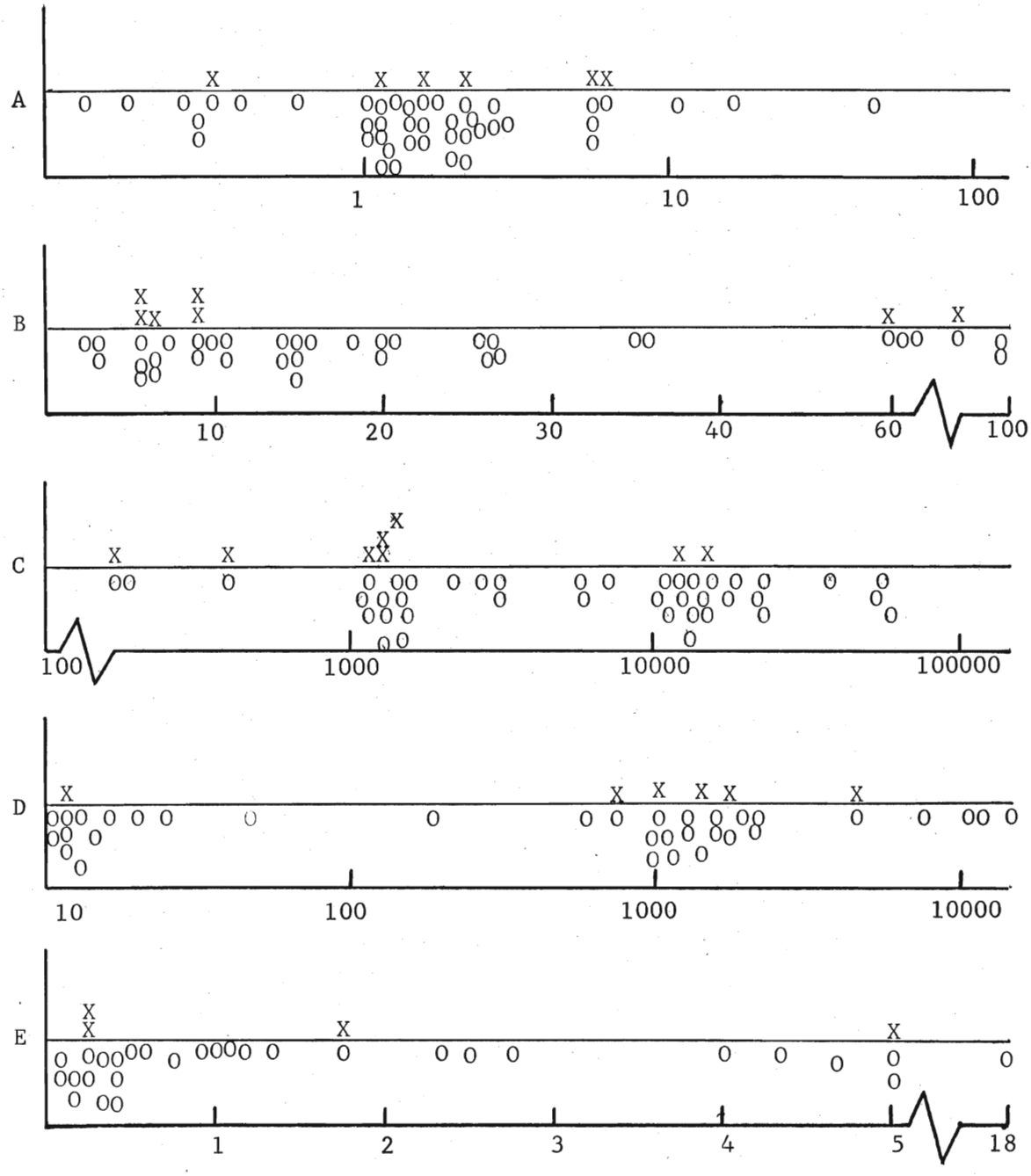
FIGURE 12 - RELATIONSHIP OF SALMONELLA ISOLATIONS FROM THE SEWAGE INFLOW AND EFFLUENT TO TEMPERATURE.

the 16.0-20.0C range. Results for the sewage effluent present a different pattern of distribution where the bulk of isolations are in the 8.0-12.0C and 12.0-16.0C ranges. Chlorination of the effluent probably does influence the distribution pattern by Salmonella elimination during the summer months reducing Salmonella isolations in the 16.1-20.0C and 20.1-24.0C ranges. Total combined isolations for both sewage sample stations contribute to a peak in levels in the 12.1-16.0C range.

E - SALMONELLA-COLIFORM RELATIONSHIP

Compared to population levels of total and faecal coliform estimations for all sample stations, the dispersion of Salmonella isolations suggested interesting differences between sample stations (Figure 13, total coliforms; Figure 14, faecal coliforms). For the sewage inflow, the dispersion of Salmonella isolations over levels of total coliforms was somewhat clumped in two ranges, $1.0x10^6$-$2.0x10^6$ and $2.0x10^7$-$3.0x10^7$. However, the dispersion of Salmonella in the inflow compared to the faecal coliform population did not show clumped distribution. Nevertheless, the majority of Salmonella isolations appeared over the lower ranges of faecal coliform levels. Interestingly, the opposite phenomenon was observed for Salmonella isolations compared to both total and faecal populations in the sewage effluent. The bulk of Salmonella isolations, over 75%, occurred during times when coliform populations were high (Table 7). Although the dispersion of Salmonella isolations over coliform population levels in Twelve Mile Creek appeared to be somewhat clumped for total coliforms, overall isolations seemed to be largely dependent on isolation attempts and were thus random. Similar random results from Lake Ontario were noted both for total and faecal coliform levels. Some clumping of Salmonella isolations was observed over

FIGURE 13 - DISPERSION OF SALMONELLA ISOLATIONS OVER THE RANGE OF TOTAL COLIFORM COUNTS FOR ALL SAMPLE STATIONS.



total coliforms x 1000

A - Lake Ontario

B - Twelve Mile Creek

C - Sewage Inflow

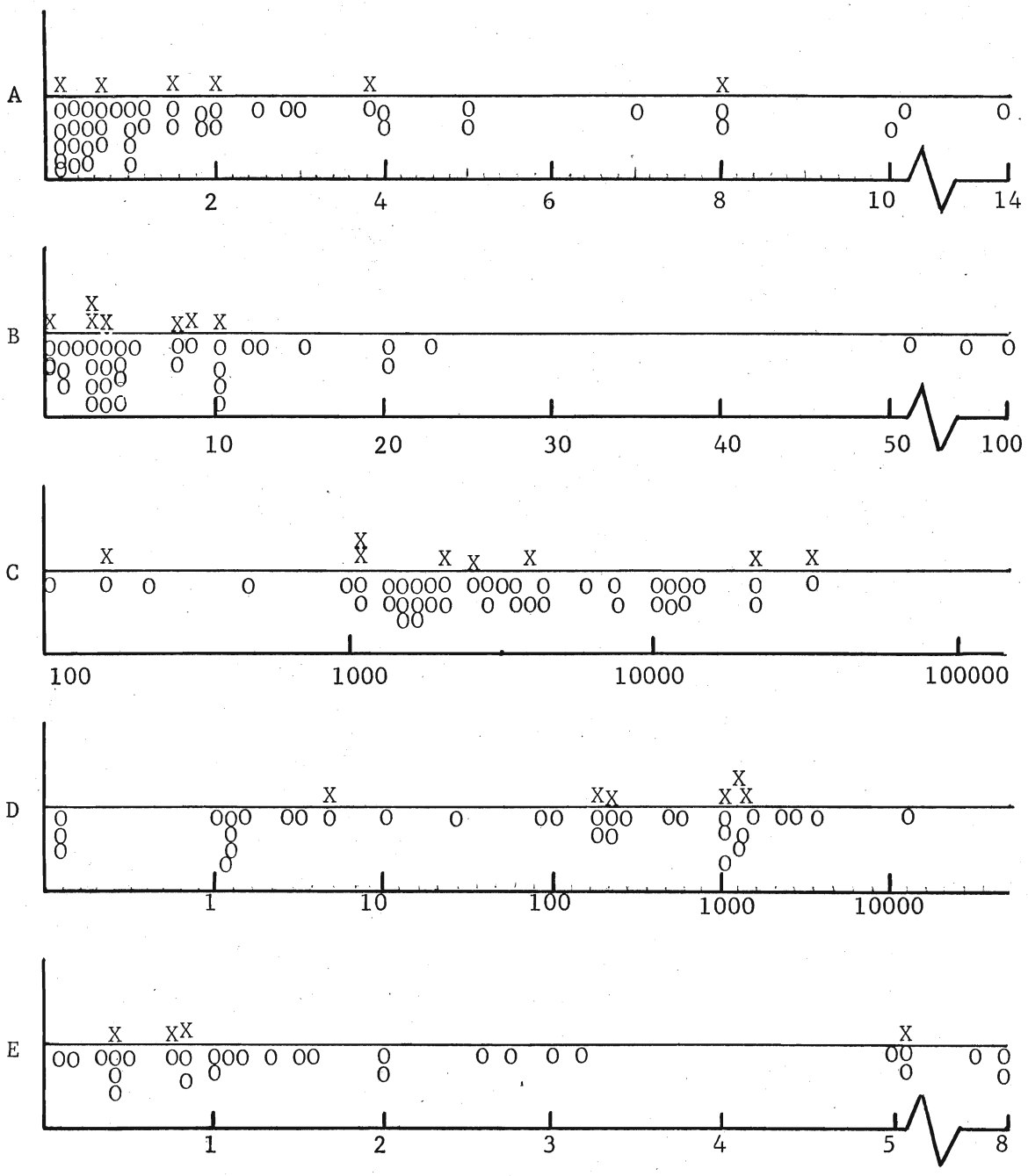
D - Sewage Effluent

E - Twenty Mile Creek

O - total coliform determination

X - Salmonella isolation

FIGURE 14 - DISPERSION OF SALMONELLA ISOLATIONS OVER THE RANGE OF FAECAL COLIFORM COUNTS FOR ALL SAMPLE STATIONS.



faecal coliforms x 100

A - Lake Ontario

B - Twelve Mile Creek

C - Sewage Inflow

D - Sewage Effluent

E - Twenty Mile Creek

O - total coliform determinatio

X - Salmonella isolation

TABLE 9 - SUMMARY OF *SALMONELLA* ISOLATE DISTRIBUTION OVER OR UNDER THE MEDIAN OF TOTAL AND FAECAL COLIFORM POPULATION LEVELS FROM ALL SAMPLE STATIONS ON SELECTED ISOLATION DATES.

Source	Total Coliform	Faecal Coliform
Lake Ontario	0	0
Twelve Mile Creek	0	0
Sewage Inflow	0	0
Sewage Effluent	+	+
Twenty Mile Creek	0	—

+ - >75% *Salmonella* isolates over the median of total or faecal coliform range

0 - random distribution

— - < 75% isolates under the median of total or faecal coliform range

lower ranges of faecal coliform levels in Twenty Mile Creek. However, Salmonella organisms were isolated on four sample days only and, therefore, little can be deduced from comparative isolation tendencies. Over 75% of the Salmonella isolations occurred above the median of total and faecal coliform population levels in the sewage effluent, while in Twenty Mile Creek, 75% of the isolations occurred below the median of faecal coliform levels.

IV DISCUSSION

The set of factors controlling population levels of enteric bacteria present in the four sample stations in the Port Dalhousie area appeared to differ for each station. This was expected, and was considered, when selection of sample sites was made, taking into account sources and composition of each body of water. In general, the sewage inflow carried primarily raw sewage and some stormwater drainage. Therefore, high levels of coliforms were expected and were regularly recovered. Unexpectedly, faecal coliform levels made up only 5.0±1.2% (from the geometric mean) of the total coliform population. By contrast, Geldreich (1968) noted that faecal coliform types normally comprised 91-97% of the coliform organisms isolated from fresh human faecal matter. It can only be assumed that various unknown factors such as industrial wastes, storm drainage and dilution were acting on coliform populations, modifying their composition. The period of water-travel time between the sewage inflow and effluent was hours, and depended upon the flow rate at the inflow. During this unspecified time, the enteric bacterial populations were subjected to the rigors of the sewage treatment process which included late spring and summer chlorination. It was shown by ANOVA and Student's t test that faecal coliform populations were significantly reduced between the sewage sample stations, whereas the total group was reduced to a lesser extent. The greater effect on the faecal coliforms was also substantiated by the reduction in the percentage composition of the faecal types from the total group between the sewage sample stations. Sewage treatment, therefore, appeared to have a greater lethal effect on

the faecal population than on non-faecal coliforms. During the spring and summer of 1972, interrupted operation of the chlorine chamber produced erratic fluctuations in faecal coliform levels. When the chlorination system was not functioning, large numbers of faecal organisms were carried directly into the harbour area of Twelve Mile Creek.

The actual water-travel time from the sewage effluent station into Twelve Mile Creek must have been only a few minutes. The flow time from the sewage outfall to the Twelve Mile Creek sample station was similarly short. Due to this brief travel time, there was little time for regrowth to occur between these sample stations. Dilution must have been a major factor affecting observed coliform levels. The chlorinated or unchlorinated effluent was quickly diluted with the large volume, fast flowing waters of Twelve Mile Creek. This acted to distribute concentrations of enteric organisms within the creek and to dilute bactericidal levels of chlorine to ineffective levels. It is unlikely that any great die off or regrowth could have occurred in Twelve Mile Creek from the sewage outfall to Lake Ontario as the distance was so short. Coleman et al. (1974) documented large increases in faecal coliform levels downstream from Edmonton in the Saskatchewan River. They attributed those increases to the large bacterial innoculum from the sewage effluent and to some regrowth of faecal coliforms in the nutrient enriched receiving waters. They found that these large populations were maintained for almost 100 miles downstream before some decline in numbers was noted. While total and faecal coliform levels were significantly lower in Twelve Mile Creek than in the sewage effluent, the percentage composition of faecal organisms to the total group was $2.3 \pm 1.4\%$ compared to $1.4 \pm 1.6\%$ in the effluent. Thus, there was no great decrease in

the percentage of faecal coliforms compared to total coliforms at both sample stations. This suggested that the population sampled in the creek had relatively the same faecal to total ratio which had been released from the sewage effluent.

In general, coliform levels remained above recommended bacteriological limitations for recreational waters of Twelve Mile Creek. The Ontario Ministry of the Environment has suggested that where ingestion is probable, recreational water can be considered impaired when the coliform and faecal coliform geometric mean density exceed 1000 and/or 100 per 100 ml, respectively, in a series of at least ten samples per month. The geometric means for total and faecal coliforms for the summer months (June, July and August) were 20,000 and 220, respectively. In fact, children were observed to swim in the creek on several occasions. It could well be that water from Twelve Mile Creek might have taken several hours, or even days, to reach the Lakeside Park beach area of Lake Ontario. Under calm conditions, the water in Twelve Mile Creek flowed from the extended piers straight out into Lake Ontario and away from the beach. Moreover, under most stormy conditions, north-westerly winds carried the bacteria laden waters of Twelve Mile Creek to the east and away from the beach. However, it was suspected that the western pier, in its state of disrepair, allowed creek water to seep through into the beach area. On rare occasions, water from Twelve Mile Creek was carried towards the beach by north-easterly winds. The possibility that creek water was reaching the beach area was suggested by comparisons of the pattern of variation of the populations shown in Figures 4 and 5. Populations of faecal coliform bacteria at both sample stations were often observed to rise and fall about the same time. For example, during November of 1971

and April, June and July of 1972, large decreases were observed for both sample stations. It was not known whether these decreases in faecal levels of Twelve Mile Creek were responsible for similar changes in levels within the lake. On the other hand, it is possible that changes in levels at both stations were controlled by some common factor. Because erratic faecal coliform populations were observed at the beach and because faecal coliforms made up 5.4±1.2% of the total group as compared to 2.3±1.4% in Twelve Mile Creek, several sewage sources were probably contributing to faecal coliform levels in the lake. Although regrowth of faecal organisms remained a possibility in the lake, low nutrient levels made it unlikely. Interestingly, large amounts of organic silt were present on the bottom of the shallow bathing waters at Lakeside Park. Hendricks and Morrison (1967) noted that extensive regrowth of enteric bacteria could occur in the micro-environment of bottom sediment where nutrients were in high concentrations even if these areas were surrounded by a relatively vast area devoid of nutrients. Any sewage pollution carried by Twelve Mile Creek was, of course, further diluted in Lake Ontario. This was evident in significant differences observed for faecal coliform levels between Twelve Mile Creek and Lake Ontario. However, levels of total coliforms between sample sites did not differ significantly. While the summer geometric mean value (32 per 100 ml) of faecal coliforms was below the maximum acceptable at 100 per 100 ml, the total coliform mean was 1300 and exceeded the bacteriological limit of 1000 per 100 ml.

Total and faecal coliform populations in Twenty Mile Creek did not show consistant levels and varied over the entire sample period. In addition to noticable variations, faecal populations made up 14.4±2.1% of the total coliform population based on the geometric mean. The relatively large

percentage of faecal coliforms regularly isolated from Twenty Mile Creek and the erratic distribution of coliform levels is suggestive of faecal matter deposition in or near the creek banks by livestock.

Of the nutrients and physical factors that were monitored during the study period, temperature was the factor that showed the most constant seasonal trends at all sample points. During the mid-winter period, January through March, 1972, water temperature for Twelve Mile Creek, Lake Ontario and Twenty Mile Creek remained near or below 2C. By contrast, the sewage inflow and effluent did not cool below 10.5C during the year. Therefore, the sewage outfall into Twelve Mile Creek was nearly 10C warmer than the receiving waters and did represent a form, although minor, of heat pollution. The possibility existed that, at least locally, the warmer sewage waters could have altered metabolic rates of some bacteria downstream of the outfall. This temperature effect on oxygen utilization by E. coli was noted by Rao and Dutka (1974) who were able to show in laboratory studies that the metabolic rate of E. coli was markedly higher at 20C than at 4C. However, some strains of natural aquatic organisms, for example Flavobacterium, had greater oxygen uptake rates at the lower temperature. Thus temperature increases might have inhibited some organisms while favoring some coliform organisms in the outfall area. That increases of temperature can influence bacterial diversity in water has been demonstrated by Guthrie et al. (1974) and Cherry et al. (1974). Seasonal trends of temperature showed that the waters of Twelve Mile Creek, Lake Ontario and Twenty Mile Creek increased above 15C only for the period of early May until early October. The two sewage stations were above this level from early May to late October.

Normally, the pH ranges of all sample stations remained within the range of 5.5-7.5 which McFeters and Stuart (1972) found to be optimal for

survival of E. coli. Dissolved oxygen and total dissolved solids did not show any seasonal trends and probably were of little consequence to the changes in population levels. Interestingly, Allen et al. (1952) found (under laboratory conditions) that aerobic conditions did favor survival of Bacterium coli (E. coli). For the short time that dissolved oxygen was measured, aerobic conditions were observed at every sample station, although the levels were usually low in the sewage inflow and effluent. This was, of course, expected.

Nutrient levels at all sample stations were, at times, detected in concentrations sufficient for increased survival or even growth of some coliform organisms (e.g. Enterobacter aerogenes) according to the study by Hendricks and Morrison (1967) using dialysis sack cultures. They showed that the aquatic environment in a clear mountain stream could not only maintain populations of enteric bacteria, but could also supply sufficient nutrients to initiate multiplication. However, they did not observe growth of enteric bacteria in the river itself, only in dialysis sack cultures. They attributed this difference between the river and the cultures to the nebulous role of stream self-purification.

Correlation and regression analysis between the physical and chemical factors and the populations of total and faecal coliforms revealed that the majority of the higher, although not significant, correlations existed for two sample stations, Lake Ontario and Twenty Mile Creek. However, the majority of the observed variation for all regression data was not due to any linear relationship. It is interesting to note that some of the better correlations existed only for faecal coliforms. If the total and faecal

coliform populations had had analogous growth or survival characteristics, then it would have been expected that both groups would show similar correlations with the independent variables, particulary with nutrients or temperature. Geldreich (1970) and McFeters et al. (1974) have noted that many coliform organisms of the total group (e.g. Enterobacter aerogenes) do not have specific growth requirements and may survive longer than most faecal coliform members. The former group may even grow in water containing minimal nutrient levels. Therefore, this adds further evidence that the low correlation between temperature and faecal coliform populations was due merely to chance. It is interesting to note that both total and faecal coliform populations showed a similar degree of correlation with phosphate P and ammonium N in Twenty Mile Creek. Although coliform organisms may have survived longer or grown in response to increased nutrient levels, it is more likely that both the coliform populations and the nutrients had been introduced into the stream in association with each other. Moreover, the regression analysis also revealed that the independent variable, phosphate P, was more highly correlated with total and faecal coliform populations in Lake Ontario. Similarly, Brasfield (1972) observed no significant correlation between phosphates and total coliforms.

At the onset of the ecological study in the Port Dalhousie area and in Twenty Mile Creek, temperature had been considered a factor likely to influence levels of coliform throughout the year. Several recent studies

had established that the survival of faecal coliforms was inversely related to temperature below 15C (McFeters and Stuart, 1972; Geldreich et al., 1968). In addition, Coleman et al. (1974) detected a positive correlation between temperature and E. coli levels. However, this was probably the result of input of faecal pollution into a cold, good-quality, mountain stream which warmed along its course onto the prairies of Alberta. By contrast, Brasfield (1972) did not find a significant correlation between temperature and total coliform levels in the Gallinas River, New Mexico. Similarly, temperature and coliform levels were not correlated except for temperature and faecal coliform levels in Twenty Mile Creek. The varied input of raw and treated sewage from many sources in the Port Dalhousie area may have disguised any temperature related influences or relationships with coliform populations. Although Twenty Mile Creek was not known to have had any sewage effluent or large amounts of raw sewage entering at least two miles upstream from the sample station, erratic levels of coliforms were observed for the entire sample period. Because of these erratic coliform population levels, and the lack of correlation between temperature and total coliforms, it is probable that the correlation between faecal coliforms and temperature was random.

Domestic sewage normally carries varying amounts of pathogenic organisms of intestinal origin which are indigenous to human and animal populations. The most common are the Salmonellae, which have a wide range of hosts among mammals, birds and reptiles (McCoy, 1963). Among Salmonella types isolated from water, several serotypes are noted for their high degree of pathogenecity for man (Thatcher and Clark, 1969). Thatcher and Clark (1969) point out that all types must be considered as serious pathogens.

Raw sewage from a city of 120,000 inhabitants normally contains large numbers and varieties of pathogenic organisms (McCoy, 1963). As expected, the sewage inflow sample station yielded the highest percentage of Salmonella isolates at 22.9%. The percentage of isolations did not differ markedly between the sewage effluent and Twelve Mile Creek. The lowest Salmonella isolation rates were obtained at the Lake Ontario and Twenty Mile Creek sample stations. It was not known how far the sample station at Twenty Mile Creek was from any major sewage source. Nevertheless, the Salmonella isolation rate was lowest of all sample stations. However, of the other four stations, Lake Ontario was the most distant from known sewage sources. For this reason, Salmonellae were expected to have been isolated at a lower rate due to dilution and die off, and this indeed was the case.

Salmonella serotype analysis revealed that ten different serotypes were isolated. This suggested that many sources, human and animal, were contributing to Salmonella populations at all sample stations. Of the ten identified, four serotypes were considered by Thatcher and Clark (1969) to be serious food poisoning organisms. These were; S. typhimurium, S. newport, S. heidelberg and S. montevideo. No one serotype, of the ten, was confined to one sample station alone. Nor was any one serotype isolated from all four sample sites in the Port Dalhousie area on the same sample day. This was not surprising, inasmuch as Salmonella would normally not have been constantly emitted in sewage. In addition to the clustered occurrence of Salmonella in sewage, dilution would also have quickly dispersed organisms to very low concentrations in Twelve Mile Creek and Lake Ontario. Identical serotypes of Salmonella were isolated from two

adjacent sites on three occasions:

S. kottbus	- Feb. 24, 1972	Twelve Mile Creek and Lake Ontario
S. muenster	- Mar. 9, 1972	sewage inflow and effluent
S. typhimurium	- May 16, 1972	sewage effluent and Twelve Mile Creek

If the three multiple isolations are not assumed to be entirely random in nature, their occurrence suggests some pollutional interrelationship between the four sample sites might be involved.

The flow-time sequence between sample stations and time differential of sampling also reduced the probability of detecting the same serotype at all sample stations. Recovery rates of Salmonella would likely have been improved if the Moore swab (Moore, Perry and Chard, 1952) had been adopted as a sampling method. Also, the newly developed polyvalent flourescent antibody screen tests (Cherry et al., 1972) would likely have improved identification rates, had they been applied. In testing 159 water specimens from a variety of surface water sources, they found FA techniques to indicate the presence of Salmonella in 63% more specimens than were positive by standard culture methods. However, some consideration must be made on the validity of these results because of some technical abnormalties of the FA technique as applied to Salmonella identification. Some non-specific cross reactions can and do result with some regularity in addition to stain reactions with both live and dead Salmonella organisms (personal communication with Mr. D. Schieman, Ph.D., Chief, Environmental Bacteriology Laboratory, Ontario Ministry of Health).

In the presenty study, seasonal distribution of Salmonella from all sample stations, except for the sewage inflow, indicated that most isolations occurred during the winter and spring of 1972. McCoy (1963) and Geldreich

(1970) attributed increased isolations of Salmonella from the sewage during the spring of the year to infectious-periods when the human population had an increased infection rate. However, epidemiological data from the Niagara Region, collected by the Epidemiology Branch of the Ontario Ministry of Health, did not show a spring increase of reported Salmonella infections. This, of course, was not conclusive evidence against a spring infectious period as many infections pass unreported. The results of Salmonella isolation rates for the untreated sewage inflow did not indicate a definite trend for winter and spring isolation increase. However, the seasonal distribution of Salmonella isolates did relate to decreased water temperature in all sample stations except the sewage inflow. Excluding sewage inflow results, 92% of all Salmonella isolates occurred when the water was 16C or below. Thus, the differences observed in Salmonella isolations at all sample stations, except for the inflow, may have been due to increased survival in cooler waters rather than greater input during the spring. A similar relationship with temperature was noted by Geldreich et al. (1968) who found that Salmonella survived longer in water held at 10C than at 20C.

In general, the Salmonella isolation rate, compared with population levels of total and faecal coliforms, showed random distribution over the ranges of coliforms except for the total and faecal coliforms in the sewage effluent and faecal coliforms in Twenty Mile Creek. In the sewage effluent, more than 75% of the Salmonella isolations occurred during periods when total and faecal populations were maximal. This finding was similar to the results of Geldreich (1970) who was able to predict the presence of Salmonella from the faecal coliform levels. His results were:

faecal coliform density per 100 ml	Salmonella detection occurrence
1 - 200	27.6%
201 - 2000	85.2%
over 2000	98.1%

Geldreich applied the Moore swab for collection and concentration of water samples. This technique might explain his success rate for Salmonella recovery.

In the present study, more than 75% of the Salmonella types isolated from Twenty Mile Creek were recovered when ranges of faecal coliforms were low. If Geldreich's findings (1970) are generally applicable, then these results must have been due to chance. The overall poor relationship between Salmonella isolations and coliform levels in the sewage inflow, Twelve Mile Creek and Lake Ontario was similar to findings by Gallagher and Spino (1967). They observed little apparent correlation between levels of total or faecal coliforms and the isolation of Salmonella from sugar beet process waters. In addition, they suggested that Salmonella might persist under conditions adverse to the survival of faecal coliforms. By comparison, McFeters et al. (1974) found similar survival characteristics were shared by faecal coliforms and some Salmonella in well water. Interestingly, in the present study, Salmonella isolations occurred more frequently during the winter and spring when faecal coliform levels decreased from high fall and early winter levels. The slight decrease of faecal coliform levels during the spring might be due to the effects of dilution by rainfall during this season. At the same time, Salmonella occurrence must have increased in the same water as their isolation rate did not similarly decline.

This study emphasizes that the factors which affect levels of enteric organisms in natural waters are still far from clear. When one considers the

wide variety of biotic, chemical and physical factors which might have been important, it is clear that freshwater bacterial ecology is a fertile field for future study. The results of this study, in comparison and contrast with the results of other recent studies, suggest that the sanitary implications developed by such a study may be applicable only to the study area. An effort should be made in future studies to incorporate parallel laboratory and field survival studies which include natural competitors and predator populations. Although some prominent water bacteriologists have noted a relationship between increased faecal coliform levels and Salmonella presence, the results of this study did not show such a relationship. However, Salmonella isolations did appear to have been related to cooler water temperatures. Due to the presence of Salmonella and high populations of total and faecal coliforms, the sanitary and recreational quality of Lake Ontario and Twelve Mile Creek at the sample sites was seriously impaired during the year of sampling.

ADDENDUM A

The selection of Twenty Mile Creek as a comparative stream to the heavy sewage and industrially polluted Twelve Mile Creek could have been improved. Several sample sites could have been chosen, one above the confluence of Twelve Mile Creek and the main stream flow from the DeCew Power Station and Beaver Creek and another immediately above the sewage outfall in Twelve Mile Creek. The source of water flowing from the DeCew Hydro Plant was the Welland Canal. Thus, the influence of sewage and industrial wastes on bacterial populations in the main stream would have been more adequately monitored during the study period and the relative bacterial contribution of each stream measured.

In addition to better sample site selection, more information on the waterflow of Twelve Mile Creek would have been useful. Data gathered by Ontario Hydro on the waterflow of the creek indicated that the waterflow varied throughout the 24 hour day. The flow rate of Twelve Mile Creek below the weir of the Royal Henley Rowing Course was approximately 4800 cfs. In addition, 10-20 cfs. were added by the sewage outfall of the St. Catharines Sewage Treatment Plant in Port Dalhousie. Heavy rain produced higher levels than given in these examples. Nightly, the total waterflow through the generating station was reduced 20-25% when the old section of the generating plant was closed. Again in the morning at 7:30-8:00a.m. the daily flow-rate was increased by opening the old section. The total maximum flowrate of both generating systems was 7500 cfs. but this level was rarely attained. It is interesting to note that with the variable flowrate in Twelve Mile

Creek a scouring effect of the bottom might have occurred which of course could have influenced bacterial levels. The sample site selection for the original study did not take this into account.

ADDENDUM B

Statistical manipulation of the regression analysis data could have been carried further for a more complete analysis. Bacterial population measurements could have been grouped into set ranges to normalize the scattered distribution of some extreme points. However, this study probably incorporated too few sample points to apply this method successfully. It might have been interesting to have computed the data for functions other than linear. Considering their lack of significance for linear regression, it is quite possible that some of the data could have filled some other functions.

REFERENCES

Allen, L. A., S. M. Pasley and M. A. Pierce. 1952. Some factors affecting the viability of fecal bacteria in water. J. Gen. Microbiol. 7: 36-43.

American Public Health Association. 1971. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. Thirteenth Edition, New York.

Bergey's Manual of Determinative Bacteriology. 1974. Eighth Edition. The Williams and Wilkins Co., Baltimore.

Bissonnette, G. K., D. G. Stuart, T. D. Goodrich and W. G. Walter. 1970. Preliminary studies of serological types of enterobacteria occurring in a closed mountain watershed. Proceedings of the Montana Academy of Sciences, 30: 66-76.

Brasfield, H. 1972. Environmental factors correlated with size of bacterial populations in a polluted stream. Appl. Microbiol. 24: 349-352.

Brezenski, F. T. and R. Russomanno. 1968. The detection and use of Salmonella in studying polluted tidal estuaries. J.W.P.C.F. 41: 725-737.

Brezenski, F. T., R. Russomanno and P. DeFalco. 1965. The occurrence of Salmonella and Shigella in post-chlorinated and non-chlorinated sewage effluents and receiving waters. Health Lab. Science, 2: 40-47.

Carruthers, E. A. (No date). The bacteria count and the geometric mean. Public Health Engineering Service, Environmental Health Information Exchange. Bulletin No. 5. Ontario.

Cassie, R. M. 1961. The correlation coefficient as an index of ecological affinities in plankton populations. Mem. Ist. Ital. Idrobiol. 13: 151-177.

Cherry, D. S., R. K. Guthrie and R. S. Harvey. 1974. Temperature influence on bacterial populations in three aquatic systems. Water Res. 8: 149-155.

Cherry, W. B., J. B. Hanks, B. M. Thomason, A. M. Murlin, J. W. Biddle and J. M. Croom. 1972. Salmonellae as an index of pollution of surface waters. Appl. Microbiol. 24: 334-340.

Claudon, D. G., D. I. Thompson, E. H. Christenson, G. W. Lawton and E. C. Dick. 1971. Prolonged Salmonella contamination of a recreational lake by run-off waters. Appl. Microbiol. 21: 875-877.

Coleman, R. N., J. N. Campbell, F. D. Cook and D. W. S. Westlake. 1974. Urbanization and the microbial content of the North Saskatchewan River. Appl. Microbiol. 27: 93-101.

Coler, R. A. and H. B. Gunner. 1969. Microbial populations as determinates in protozoan succession. Water Res. 3: 149-156.

Deaner, D. G. and K. D. Kerri. 1969. Regrowth of fecal coliforms. J.A.W.W.A. 9: 465-468.

Duncan, D. W. and W. E. Razzell. 1972. Klebsiella biotypes among coliforms isolated from forest environments and farm produce. Appl. Microbiol. 24: 933-938.

Dutka, B. J. 1972. Technical and practical considerations in the microbial contamination of water. II. Coliforms: An inadequate index of water quality. Presented at the 40th Annual Meeting of Laboratory Division of the Canadian Public Health Association.

Dutka, B. J., J. B. Bell, P. Collins and J. Popplow. 1969. A bacteriological study of the Rainy River, conducted for the Advisory Board on Water Pollution, Rainy River and Lake of the Woods, International Joint Commission. Dept. of N. H. and W., Public Health Engineering Division. Manuscript Report No. KR. 69-1.

Dutka, B. J., A. S. Y. Chau and J. Coburn. 1974. Relationship between bacterial indicators of water pollution and fecal sterols. Water Res. 8: 1047-1055.

Edwards, P. R. and W. H. Ewing. 1960. Identification of the Enterobacteriaceae. Burgess Pub. Co., Minneapolis.

Edwards, P. R. and W. H. Ewing. 1972. Identification of the Enterobacteriaceae. Burgess Pub. Co., Minneapolis.

Evans, F. L., E. E. Geldreich, S. R. Weibel and G. G. Robeck. 1968. Treatment of urban stormwater run-off. J.W.P.C.F. 40: 162-170.

Fair, J. F. and S. M. Morrison. 1967. Recovery of bacterial pathogens from high quality surface waters. Water Resources Res. 3: 799-803.

Gallagher, T. P. and D. F. Spino. 1968. The significance of numbers of coliform bacteria as an indicator of enteric pathogens. Water Res. 2: 169-175.

Geldreich, E. E. 1970. Applying bacteriological parameters to recreational water quality. J.A.W.W.A. 62: 113-120.

Geldreich, E. E. 1972. Buffalo Lake recreational water quality: A study in bacteriological data interpretation. Water Res. 6: 913-924.

Geldreich, E. E., L. C. Best, B. A. Kenner and D. J. Van Donsel. 1968. The bacteriological aspects of stormwater pollution. J.W.P.C.F. 40: 1861-1872.

Geldreich, E. E., H. L. Jeter and J. A. Winter. 1967. Technical considerations in applying the membrane filter procedure. Health Lab. Science, 4: 113-125.

Greenberg, A. E. and H. J. Ongerth. 1966. Salmonellosis in Riverside, California. J.A.W.W.A. 58: 1145-1150.

Grunnet, K. and B. B. Nielsen. 1969. Salmonella types isolated from the Gulf of Aarhus compared with types from infected human beings, animals and feed products in Denmark. Appl. Microbial. 18: 985-990.

Guthrie, R. K., D. S. Cherry and R. N. Ferebee. 1974. A comparison of thermal loading effects on bacterial populations in polluted and non-polluted aquatic systems. Water Res. 8: 143-148.

Hendricks, C. W. 1974. Bdellovibrio bacterivorus-Escherichia coli interactions in continous culture of river water. Environmental Letters, 7: 311-319.

Hendricks, C. W. and S. M. Morrison. 1967. Multiplication and growth of selected enteric bacteria in clear mountain stream water. Water Res. 1: 567-576.

Joyce, G. and H. H. Weiser. 1967. Survival of enteroviruses and bacteriophage in farm pond waters. J.A.W.W.A. 59: 491-501.

Kuznetsov, S. I. 1968. Recent studies of the role of microorganisms in the cycling of substance in lakes. Limnology and Oceanography, 8: 211-223.

Leclerc, E. 1963. Self-purification of fresh water streams as affected by temperature and by the content of oxygen, nitrogen and other substances. Int. J. Air and Water Pollut. 7: 357-365.

Matusiak, K., R. Chróst and A. Krzywicka. 1971. Bacterial microflora in Chlorella vulgaris culture. Acta Microbiologica Polonica Ser. B. 3: 189-193.

McCoy, J. H. 1963. Salmonella in crude sewage effluent and sewage polluted natural waters. Int. J. Air and Water Pollut. 7: 597-611.

McFeters, G. A., G. K. Bissonnette, J. J. Jezeski, C. A. Thompson and D. G. Stuart. 1974. Comparative survival of indicator bacteria and enteric pathogens in well water. Appl. Microbiol. 27: 823-829.

McFeters, G. A. and D. G. Stuart. 1972. Survival of coliform bacteria in natural waters: Field and laboratory studies with membrane-filter chambers. Appl. Microbiol. 24: 805-811.

McGrew, S. B. and M. F. Mallette. 1961. Energy maintenance in Escherichia coli. J. Bact. 83: 844-850.

Miner, J. R., L. R. Fina and C. Piatt. 1967. Salmonella infantis in cattle feedlot run-off. Appl. Microbiol. 15: 627-628.

Moore, B. C., L. Perry and S. T. Chard. 1952. A survey by the sewage swab method of latent enteric infection in an urban area. J. Hyg. (Cambridge) 50: 137-156.

Ontario Ministry of the Environment. 1973. Guidelines and Criteria for Water Quality Management.

Park, D. 1972. On the ecology of heterotrophic microorganisms in fresh-water. Trans. Br. mycol. Soc. 58: 291-299.

Petersen, N. J. and J. R. Boring III. 1960. A study of coliform densities and Escherichia coli serotypes in two mountain streams. Amer. J. Epid. 7: 134-140.

Post, F. J. 1970. Ecology of selected bacteria in a small intermittent sewage pond. Water Res. 4: 341-351.

Purdy, W. C. and C. T. Butterfield. 1917. The effect of plankton animals upon bacterial death-rates. Amer. J. Pub. H. Assoc. Oct: 499-506.

Rao, S. S. and B. J. Dutka. 1974. Influence of temperature on lake bacterial activity. Water Res. 8: 525-528.

Seligman, R. and R. Reitler. 1965. Enteropathogens in water with low Escherichia coli titer. J.A.W.W.A. 57: 1572-1574.

Singh, B. N. 1945. The selection of bacterial food by soil amoebae and the toxic effects of bacterial pigments and other products on soil protozoa. Brit. J. Exp. Path. 26: 316-325.

Slanetz, L. W., C. H. Bartley and T. G. Metcalf. 1964. Correlation of coliform and fecal streptococcal indices with the presence of Salmonellae and enteric viruses in sea water and shellfish. Adv. in Water Pollut. Res. Proc. 2nd Int. Conf. on Water Pollut. Res., Tokyo, 27-37.

Spino, D. F. 1966. Elevated-temperature technique for the isolation of Salmonella from streams. Appl. Microbiol. 14: 591-596.

Stanier, R. Y., M. Doudoroff and E. A. Adelberg. 1970. The Microbial World. Prentice-Hall Inc. Englewood Cliffs, New Jersey.

Steel, R. G. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Pub., Toronto.

Stuart, D. G., G. K. Bissonnette, T. D. Goodrich and W. G. Walter. 1971. Effects of multiple use on water quality of high mountain watersheds: Bacteriological invertigations of mountain streams. Appl. Microbiol. 22: 1048-1054.

Thatcher, E. S. and D. S. Clark. 1969. Microorganisms in Foods. U. of Toronto Press, Toronto.

Van Donsel, D. J. and E. E. Geldreich. 1971. Relationships of Salmonellae to fecal coliforms in bottom sediments. Water Res. 5: 1079-1087.

Van Donsel, D. J., E. E. Geldreich and N. A. Clarke. 1967. Seasonal variations in survival of indicator bacteria in soil and their contribution to stormwater pollution. Appl. Microbiol. 15: 1362-1370.

Wright, R. T. and J. E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology, 47: 447-464.

Wun, C. K., J. R. Cohen and W. Litsky. 1972. Evaluation of plating media and temperature parameters in the isolation of selected enteric pathogens. Health Lab. Science. Amer. J. Pub. H. Assoc. 3: 225-232.

APPENDIX I

LIST OF MEDIA

Difco

Medium	Difco Catalogue Number
Lysine Decarboxylase Broth	0215
Hektoen Enteric Agar	0353
MRUP Medium	0016
m-FC Broth Base	0883
Salmonella-Shigella (SS) Agar	0074
Simmon's Citrate Agar	0091
Selenite Cystine Broth	0687
Tetrathionate Broth Base	0104
TSI (Triple Sugar Iron) Agar	0265
Urea Agar Base	0283
MacConkey's Agar	0075
Endo Agar LES	0736
Brilliant Green Broth	0494
Motility Medium S	0761
Dextrose	B155
DNase Test Agar	0632
XLD Agar	0788
Malonate Broth	0395
B.G. Sulfa Agar	0717
Bacto Agar	0140
Ornithine	0181-13
Levine EMB Agar	0005
Arabinose	B159
Xylose	0181-12
Decarboxylase Medium Base-Moeller	0872

BBL

Brain-Heart Infusion	11059
EC Broth	11187
m-FC Broth	11365

APPENDIX II

TABLE 10 - SEWAGE INFLOW RESULTS (Sample Station C).

Date	Temp °C	DO*	pH	TDS*	NO_3-N*	NH_4-N*	Total PO_4-P*
Aug 14/71	23.0	2.5	7.4	600	0.2	4.0	0.67
Aug 24/71	23.0	3.0	7.5	500	0.2	3.0	1.00
Sep 10/71	22.5	2.7	7.2	700	0.3	3.5	0.93
Sep 17/71	22.0	3.0	7.2	400	0.2	3.0	0.83
Sep 23/71	22.7	5.0	7.3	700	0.2	3.6	0.67
Sep 30/71	22.0	5.2	8.0	700	1.5	9.6	0.73
Oct 4/71	22.0	4.8	7.4	600	1.0	9.2	0.67
Oct 11/71	22.3	4.2	7.6	500	1.6	12.0	0.87
Oct 18/71	22.5	4.0	7.8	300	1.0	11.0	1.00
Oct 24/71	22.5	3.2	7.6	200	1.2	6.0	0.67
Oct 29/71	21.0	3.6	7.8	400	1.4	8.4	1.20
Nov 4/71	20.5	3.2	7.8	600	1.5	18.0	2.33
Nov 11/71	19.5	4.0	7.7	500	1.6	12.0	1.60
Nov 25/71	18.0	3.0	7.8	500	1.7	18.0	1.67
Dec 1/71	17.0	4.4	7.9	400	1.9	14.0	2.00
Dec 10/71	16.0	7.0	7.7	500	2.0	16.0	2.33
Dec 22/71	14.0	4.5	8.3	800	2.0	20.0	2.33
Dec 29/71	13.0	4.0	8.0	400	2.2	20.0	2.67
Jan 12/72	13.0	4.0	7.9	1000	2.4	18.0	2.67
Jan 20/72	10.5	6.5	8.0	400	0.5	5.0	1.87
Jan 27/72	14.0	4.5	8.1	900	1.4	6.4	2.50
Feb 6/72	12.5	3.5	7.9	1300	1.5	22.0	2.33
Feb 16/72	13.0	5.0	8.3	700	1.6	12.0	2.17
Feb 24/72	12.0	4.5	8.0	1000	1.8	14.4	2.00
Mar 9/72	11.0	3.0	7.2	800	3.0	16.0	2.33
Mar 28/72	11.0	3.5	7.9	800	2.0	14.0	2.40
Apr 18/72	12.0	3.5	7.8	600	1.9	6.0	2.33
Apr 27/72	12.5	4.5	7.7	1000	0.8	6.0	1.67
May 4/72	13.5	4.0	8.0	200	1.0	4.0	1.33
May 12/72	14.0	---≠	7.9	600	1.2	6.0	1.83
May 16/72	15.0	---	5.8	1000	0.8	4.4	2.67
May 26/72	16.0	---	7.8	400	1.4	8.2	1.67
Jun 2/72	17.0	---	8.0	600	2.4	10.0	1.67
Jun 13/72	17.5	---	8.1	300	2.0	10.4	1.93
Jun 21/72	21.0	---	7.7	400	2.0	13.0	1.70
Jun 28/72	19.0	---	7.7	400	1.3	12.0	2.00
Jul 6/72	21.0	---	7.8	300	2.2	10.2	1.53
Jul 14/72	21.0	---	7.7	300	2.0	12.0	1.67
Jul 20/72	21.5	---	7.7	300	4.0	11.0	1.67
Jul 25/72	21.5	---	7.8	400	2.5	10.4	2.00
Aug 3/72	21.5	---	7.8	400	2.0	12.0	2.07

* - ppm

≠ - no oxygen readings taken

TABLE 10 (continued).

Date	Total Coliforms Per 100 ml	Faecal Coliforms Per 100 ml
Aug 14/71	3.6×10^7	4.1×10^6
Aug 24/71	2.3×10^7	1.5×10^6
Sep 10/71	2.8×10^7	2.6×10^6
Sep 17/71	2.1×10^7	2.1×10^6
Sep 23/71	2.7×10^7	1.8×10^6
Sep 30/71	8.0×10^6	8.0×10^5
Oct 4/71	2.3×10^7	7.0×10^5
Oct 11/71	4.2×10^7	1.0×10^6
Oct 18/71	6.2×10^7	9.0×10^5
Oct 24/71	8.0×10^7	3.0×10^5
Oct 29/71	1.6×10^7	9.2×10^5
Nov 4/71	2.3×10^7	4.0×10^6
Nov 11/71	1.2×10^7	6.0×10^5
Nov 25/71	3.2×10^7	5.1×10^5
Dec 1/71	8.0×10^7	3.2×10^5
Dec 10/71	4.0×10^7	1.0×10^5
Dec 22/71	8.0×10^7	1.7×10^6
Dec 29/71	2.6×10^7	4.0×10^5
Jan 12/72	5.0×10^6	7.0×10^5
Jan 20/72	4.2×10^6	2.4×10^5
Jan 27/72	1.7×10^6	2.7×10^5
Feb 6/72	2.2×10^7	5.8×10^6
Feb 16/72	5.5×10^6	2.3×10^5
Feb 24/72	3.1×10^7	1.1×10^6
Mar 9/72	6.8×10^5	3.0×10^4
Mar 28/72	2.4×10^6	2.2×10^5
Apr 18/72	2.4×10^6	3.1×10^5
Apr 27/72	2.5×10^6	5.3×10^5
May 4/72	2.0×10^6	1.4×10^5
May 12/72	2.8×10^6	2.6×10^5
May 16/72	3.7×10^5	1.5×10^5
May 26/72	1.2×10^6	3.5×10^5
Jun 2/72	5.6×10^6	6.0×10^5
Jun 13/72	2.3×10^6	6.4×10^5
Jun 21/72	1.4×10^6	4.6×10^5
Jun 28/72	4.0×10^5	8.0×10^2
Jul 6/72	2.4×10^6	4.2×10^4
Jul 14/72	9.0×10^6	7.0×10^4
Jul 20/72	1.4×10^7	3.8×10^5
Jul 25/72	8.0×10^6	5.8×10^5
Aug 3/72	1.1×10^7	3.2×10^5

TABLE 11 - SEWAGE EFFLUENT RESULTS (Sample Station D).

Date	Temp °C	DO*	pH	TDS*	NO_3-N*	NH_4-N*	Total PO_4-P*
Sep 23/71	21.6	0.9	7.2	700	1.2	12.0	0.07
Sep 30/71	22.0	2.8	7.4	600	0.2	12.0	0.07
Oct 4/71	21.4	1.6	7.3	800	0.2	12.6	0.07
Oct 11/71	21.7	2.0	7.4	700	0.2	18.0	1.67
Oct 18/71	22.0	2.2	7.5	600	1.2	20.0	2.00
Oct 23/71	22.7	2.4	7.2	500	1.0	9.0	1.67
Nov 4/71	20.0	0.8	7.4	500	1.2	24.0	2.00
Dec 10/71	15.0	8.0	7.4	600	1.4	20.0	2.00
Dec 22/71	15.0	5.0	7.8	900	1.5	18.0	2.33
Dec 29/71	14.5	4.5	7.7	700	0.2	19.0	2.00
Jan 12/72	13.0	6.0	8.1	700	1.6	20.0	2.33
Jan 20/72	12.0	6.0	8.1	600	0.5	8.0	2.13
Jan 27/72	13.0	4.5	7.8	900	2.0	12.0	3.33
Feb 6/72	13.0	4.5	7.9	1300	3.0	16.0	3.33
Feb 16/72	12.0	5.5	8.4	600	4.0	12.0	2.33
Feb 24/72	12.0	5.5	7.9	900	1.5	24.0	2.00
Mar 9/72	11.0	3.0	7.7	500	1.0	15.2	0.93
Mar 28/72	11.0	3.0	7.9	800	2.0	14.0	1.67
Apr 18/72	12.5	3.0	7.7	600	2.0	9.6	1.33
Apr 27/72	13.0	2.5	7.5	500	3.0	7.2	2.13
May 4/72	14.0	---‡	7.8	200	4.0	5.6	2.27
May 16/72	16.0	---	7.7	700	2.0	7.8	2.33
May 28/72	18.0	---	7.8	500	2.5	9.0	2.10
Jun 2/72	18.5	---	7.8	400	0.4	10.5	2.07
Jun 13/72	19.0	---	7.5	200	0.8	13.2	2.67
Jun 21/72	21.5	---	7.5	400	2.5	14.6	2.33
Jun 28/72	20.0	---	7.8	300	2.0	8.0	2.47
Jul 6/72	20.5	---	7.8	200	2.0	3.0	2.00
Jul 13/72	21.0	---	7.6	300	1.6	13.0	2.33
Jul 14/72	22.0	---	7.4	400	1.8	12.0	2.53
Jul 20/72	22.5	---	7.5	400	2.0	12.0	2.33
Jul 25/72	23.0	---	7.4	400	1.8	10.0	2.67
Aug 3/72	23.0	---	7.4	300	1.6	11.0	2.27

* - ppm

‡ - no oxygen readings taken

TABLE 11 (continued).

Date	Total Coliforms Per 100 ml	Faecal Coliforms Per 100 ml
Sep 23/71	3.6×10^5	4.9×10^3
Sep 30/71	8.0×10^5	2.0×10^2
Oct 4/71	1.2×10^6	1.2×10^5
Oct 11/71	1.6×10^7	1.6×10^4
Oct 18/71	2.0×10^6	4.2×10^4
Oct 23/71	3.0×10^6	3.0×10^4
Nov 4/71	6.5×10^7	4.2×10^5
Dec 10/71	4.0×10^6	9.0×10^3
Dec 22/71	1.1×10^7	2.0×10^6
Dec 29/71	7.0×10^6	1.0×10^5
Jan 12/72	3.0×10^6	6.1×10^5
Jan 20/72	3.9×10^6	5.0×10^4
Jan 27/72	1.0×10^6	5.0×10^5
Feb 6/72	1.1×10^6	1.8×10^5
Feb 16/72	3.8×10^6	7.0×10^4
Feb 24/72	1.0×10^6	3.0×10^4
Mar 9/72	2.5×10^6	3.5×10^5
Mar 28/72	3.3×10^6	1.9×10^5
Apr 18/72	9.0×10^6	8.0×10^4
Apr 27/72	9.0×10^5	4.3×10^4
May 4/72	1.4×10^6	1.5×10^5
May 16/72	1.7×10^4	7.0×10^2
May 28/72	1.8×10^4	5.4×10^2
Jun 2/72	1.7×10^4	1.6×10^2
Jun 13/72	5.7×10^3	0
Jun 21/72	1.8×10^4	1.3×10^3
Jun 28/72	2.1×10^6	4.7×10^2
Jul 6/72	2.9×10^4	0
Jul 13/72	1.6×10^4	1.8×10^2
Jul 14/72	1.3×10^3	0
Jul 20/72	3.6×10^4	1.0×10^2
Jul 25/72	4.4×10^4	2.8×10^2
Aug 3/72	2.6×10^4	1.6×10^2

TABLE 12 - TWELVE MILE CREEK RESULTS (Sample Station B).

Date	Temp °C	DO*	pH	TDS*	NO_3-N*	NH_4-N*	Total PO_4-P*
Sep 22/71	17.9	7.2	7.8	500	0.20	0.2	0.07
Sep 30/71	18.7	7.7	7.7	400	0.22	0.2	0.07
Oct 4/71	18.2	7.5	7.8	400	0.14	0.2	0.07
Oct 11/71	18.0	7.6	8.0	500	0.17	0.2	0.07
Oct 18/71	17.5	7.5	7.9	1000	0.20	0.2	0.07
Oct 23/71	17.2	7.4	8.0	200	0.28	0.2	0.13
Nov 4/71	15.4	9.3	8.0	400	0.13	0.2	0.07
Nov 25/71	7.3	10.2	8.2	1400	0.14	0.2	0.07
Dec 10/71	9.0	8.5	8.0	1500	0.24	0.3	0.13
Dec 22/71	5.0	8.0	8.1	300	0.34	0.3	0.20
Dec 29/71	3.5	9.0	8.0	1200	0.20	0.2	0.17
Jan 12/72	4.0	5.0	7.7	500	0.21	0.2	0.13
Jan 20/72	3.0	8.5	8.1	300	0.18	0.4	0.10
Jan 27/72	4.0	6.5	8.2	700	0.40	0.2	0.27
Feb 6/72	3.0	5.5	8.1	300	0.24	0.4	0.10
Feb 16/72	3.0	7.0	8.1	300	0.18	0.3	0.13
Feb 24/72	3.0	8.0	8.2	600	0.20	0.2	0.13
Mar 9/72	2.0	4.5	8.1	200	0.23	0.6	0.17
Mar 28/72	3.0	4.0	8.1	400	0.26	0.4	0.33
Apr 18/72	4.5	4.0	8.1	200	0.19	0.2	0.13
Apr 27/72	4.0	4.0	8.0	300	0.20	0.2	0.10
May 4/72	6.0	---†	8.1	400	0.20	0.2	0.10
May 12/72	7.5	---	8.3	400	0.22	0.2	0.13
May 16/72	8.0	---	8.1	500	0.32	0.2	0.13
May 22/72	11.5	---	8.2	400	0.36	0.2	0.23
May 28/72	10.5	---	8.2	400	0.20	0.2	0.10
Jun 4/72	11.0	---	8.0	300	0.21	0.2	0.07
Jun 13/72	11.5	---	8.1	200	0.19	0.2	0.07
Jun 21/72	17.5	---	8.0	300	0.20	0.3	0.07
Jun 28/72	15.0	---	8.0	200	0.16	0.2	0.07
Jul 6/72	18.0	---	8.1	300	0.13	0.3	0.07
Jul 14/72	20.0	---	8.1	200	0.14	0.2	0.10
Jul 20/72	21.0	---	8.0	200	0.16	0.2	0.13
Jul 25/72	21.5	---	8.0	300	0.17	0.2	0.13
Aug 3/72	21.5	---	8.1	400	0.16	0.2	0.07

* - ppm

† - no oxygen readings taken

TABLE 12 (continued).

Date	Total Coliforms Per 100 ml	Faecal Coliforms Per 100 ml
Sep 22/71	2.7×10^{4}	1.3×10^{3}
Sep 30/71	3.0×10^{6}	5.0×10^{2}
Oct 4/71	2.6×10^{4}	8.0×10^{2}
Oct 11/71	2.5×10^{4}	1.5×10^{3}
Oct 18/71	3.6×10^{4}	2.3×10^{3}
Oct 23/71	4.6×10^{5}	1.0×10^{3}
Nov 4/71	6.9×10^{4}	8.0×10^{2}
Nov 25/71	1.0×10^{4}	1.8×10^{2}
Dec 10/71	7.0×10^{3}	4.0×10^{2}
Dec 22/71	1.6×10^{4}	1.0×10^{4}
Dec 29/71	3.5×10^{3}	1.0×10^{3}
Jan 12/72	2.6×10^{3}	1.0×10^{3}
Jan 20/72	3.3×10^{3}	2.7×10^{2}
Jan 27/72	9.0×10^{3}	9.0×10^{2}
Feb 6/72	9.0×10^{3}	1.0×10^{3}
Feb 16/72	1.1×10^{4}	4.0×10^{2}
Feb 24/72	5.0×10^{4}	2.4×10^{2}
Mar 9/72	2.7×10^{4}	2.0×10^{3}
Mar 28/72	6.0×10^{3}	20
Apr 18/72	6.1×10^{4}	6.0×10^{3}
Apr 27/72	1.5×10^{4}	2.6×10^{2}
May 4/72	5.4×10^{3}	4.0×10^{2}
May 12/72	2.0×10^{4}	4.0×10^{2}
May 16/72	5.7×10^{3}	2.6×10^{2}
May 22/72	1.5×10^{4}	1.2×10^{3}
May 28/72	6.2×10^{3}	3.4×10^{2}
Jun 4/72	2.1×10^{4}	3.0×10^{2}
Jun 13/72	3.5×10^{4}	1.1×10^{2}
Jun 21/72	1.5×10^{4}	2.0×10^{3}
Jun 28/72	2.0×10^{4}	20
Jul 6/72	1.8×10^{4}	3.5×10^{2}
Jul 14/72	1.4×10^{4}	0
Jul 20/72	6.7×10^{4}	4.0×10^{2}
Jul 25/72	1.4×10^{4}	8.0×10^{3}
Aug 3/72	1.1×10^{4}	90

TABLE 13 - LAKE ONTARIO RESULTS (Sample Station A).

Date	Temp °C	DO*	pH	TDS*	NO_3-N*	NH_4-N*	Total PO_4-P*
Aug 14/71	20.0	7.0	7.9	400	0.20	0.2	0.07
Aug 24/72	19.5	7.4	7.8	600	0.15	0.2	0.07
Sep 10/71	20.0	6.0	7.7	500	0.17	0.2	0.07
Sep 17/71	20.0	5.8	7.8	100	0.22	0.2	0.07
Sep 23/71	17.7	8.2	7.2	400	0.16	0.2	0.17
Sep 30/71	18.2	5.6	7.7	400	0.22	0.2	0.13
Oct 4/71	17.8	5.8	7.8	500	0.24	0.2	0.20
Oct 11/71	17.6	6.0	7.8	500	0.33	0.2	0.23
Oct 18/71	17.2	6.8	8.0	600	0.35	0.2	0.23
Oct 23/71	16.0	7.6	7.8	200	0.28	0.2	0.23
Oct 29/71	14.5	7.8	8.1	400	0.26	0.2	0.17
Nov 4/71	11.0	9.0	8.2	500	0.24	0.2	0.26
Nov 11/71	12.0	9.5	8.1	500	0.42	0.2	0.33
Nov 24/71	6.3	10.2	8.1	500	0.26	0.2	0.13
Dec 1/71	9.0	8.0	8.0	600	0.19	0.2	0.23
Dec 10/71	10.0	7.0	7.0	600	0.22	0.2	0.26
Dec 22/71	6.0	6.5	8.1	400	0.28	0.2	0.33
Dec 27/71	4.5	8.0	8.2	600	0.32	0.2	0.33
Jan 12/72	5.0	5.5	8.0	500	0.34	0.3	0.40
Jan 20/72	4.0	8.5	8.0	200	0.24	0.3	0.17
Jan 27/72	3.0	6.0	7.9	700	0.22	0.2	0.17
Feb 6/72	3.0	5.0	8.0	200	0.22	0.2	0.17
Feb 16/72	3.0	4.0	8.1	200	0.18	0.3	0.07
Feb 24/72	3.0	7.5	8.0	400	0.28	0.4	0.23
Mar 9/72	2.0	4.5	7.9	500	0.30	0.4	0.20
Mar 28/72	2.5	3.5	7.8	600	0.32	0.4	0.26
Apr 11/72	4.0	4.5	7.9	400	0.40	0.3	0.23
Apr 18/72	4.0	4.5	7.9	300	0.38	0.2	0.20
Apr 27/72	5.5	4.0	8.0	300	0.24	0.3	0.13
May 4/72	8.0	---∓	8.3	300	0.22	0.2	0.13
May 12/72	8.5	---	8.2	400	0.19	0.2	0.10
May 16/72	10.0	---	8.4	400	0.34	0.2	0.20
May 22/72	13.5	---	8.4	400	0.30	0.2	0.17
May 28/72	12.5	---	8.4	600	0.21	0.4	0.13
Jun 2/72	14.0	---	8.3	300	0.20	0.4	0.13
Jun 13/72	15.0	---	8.2	100	0.18	0.4	0.10
Jun 21/72	16.0	---	8.3	200	0.20	0.2	0.07
Jun 28/72	13.0	---	8.1	300	0.18	0.4	0.07
Jul 5/72	16.0	---	8.2	100	0.12	0.2	0.07
Jul 14/72	18.0	---	8.2	200	0.14	0.2	0.07
Jul 20/72	18.5	---	8.1	100	0.15	0.2	0.07
Jul 25/72	18.5	---	8.1	100	0.15	0.2	0.07
Aug 3/72	19.5	---	8.2	100	0.16	0.2	0.07

* - ppm

∓ - no oxygen readings taken

TABLE 13 (continued).

Date	Total Coliforms Per 100 ml	Faecal Coliforms Per 100 ml
Aug 14/71	2.1×10^3	90
Aug 24/71	1.6×10^3	65
Sep 10/71	2.5×10^3	1.1×10^2
Sep 17/71	5.1×10^3	2.9×10^2
Sep 23/71	4.3×10^2	0
Sep 30/71	7.0×10^4	1.4×10^3
Oct 4/71	4.0×10^3	8.0×10^2
Oct 11/71	3.8×10^3	1.2×10^3
Oct 18/71	4.3×10^3	7.0×10^2
Oct 23/71	3.9×10^3	4.0×10^2
Oct 29/71	3.8×10^3	1.8×10^2
Nov 4/71	5.1×10^3	1.0×10^2
Nov 11/71	1.3×10^3	40
Nov 24/71	1.2×10^3	10
Dec 1/71	2.8×10^3	2.0×10^2
Dec 10/71	8.0×10^3	1.1×10^2
Dec 22/71	1.5×10^3	1.8×10^2
Dec 27/71	1.0×10^3	50
Jan 12/72	3.2×10^3	2.5×10^2
Jan 20/72	1.7×10^3	4.0×10^2
Jan 27/72	4.0×10^3	8.0×10^2
Feb 6/72	1.5×10^4	5.1×10^2
Feb 16/72	8.0×10^3	5.1×10^2
Feb 24/72	7.9×10^3	3.9×10^2
Mar 9/72	2.9×10^4	1.1×10^3
Mar 28/72	4.8×10^3	1.1×10^2
Apr 11/72	3.4×10^3	1.2×10^2
Apr 18/72	4.0×10^3	2.1×10^2
Apr 27/72	1.8×10^3	1.0×10^2
May 4/72	1.5×10^3	1.5×10^2
May 12/72	8.0×10^3	1.6×10^2
May 16/72	5.4×10^2	20
May 22/72	6.2×10^2	30
May 28/72	5.2×10^2	50
Jun 2/72	1.5×10^2	60
Jun 13/72	2.9×10^3	30
Jun 21/72	4.8×10^2	10
Jun 28/72	2.7×10^2	0
Jul 5/72	8.0×10^2	35
Jul 14/72	1.8×10^3	30
Jul 20/72	2.5×10^3	20
Jul 25/72	2.4×10^3	3.0×10^2
Aug 3/72	1.2×10^3	42

TABLE 14 - TWENTY MILE CREEK RESULTS (Sample Station E).

Date	Temp °C	DO*	pH	TDS*	NO_3-N*	NH_4-N*	Total PO_4-P*
Sep 17/71	18.0	4.0	7.8	300	0.18	0.2	0.17
Sep 23/71	14.0	6.3	8.0	1000	0.24	0.2	0.17
Sep 30/71	16.0	4.2	7.7	700	0.36	0.2	0.17
Oct 4/71	14.5	3.8	7.7	800	0.26	0.2	0.17
Oct 14/71	14.2	5.6	7.6	900	0.28	0.3	0.20
Oct 21/71	15.0	5.2	7.6	1000	0.30	0.3	0.23
Oct 28/71	17.0	4.5	7.5	900	0.25	0.2	0.13
Nov 3/71	13.7	4.0	8.0	1200	0.23	0.5	0.20
Nov 25/71	5.0	9.2	8.1	500	0.18	0.5	0.20
Dec 10/71	7.0	6.5	8.0	800	0.16	0.5	0.23
Dec 16/71	9.0	7.3	7.4	700	0.17	0.8	0.27
Jan 14/72	3.5	5.0	7.4	700	0.17	0.7	0.27
Jan 21/72	2.0	6.0	7.4	600	0.13	1.0	0.17
Apr 6/72	6.0	3.0	7.4	500	0.12	0.6	0.17
Apr 13/72	8.5	5.5	7.5	400	0.18	0.5	0.20
Apr 21/72	11.0	6.0	7.4	500	0.20	0.5	0.17
May 5/72	13.5	---∓	7.6	600	0.20	0.3	0.13
May 15/72	18.0	---	7.7	1000	0.32	0.2	0.13
May 22/72	21.0	---	7.8	700	0.44	0.4	0.10
Jun 2/72	21.5	---	7.8	600	0.62	0.3	0.10
Jun 9/72	21.5	---	7.9	300	0.38	0.1	0.07
Jun 14/72	22.0	---	8.1	400	0.22	0.2	0.07
Jun 21/72	22.0	---	7.9	200	0.34	0.2	0.07
Jun 28/72	23.0	---	8.0	500	0.26	0.1	0.10
Jul 5/72	23.5	---	7.8	600	0.17	0.1	0.13
Jul 18/72	24.0	---	8.1	500	0.18	0.1	0.13
Jul 20/72	24.5	---	8.0	300	0.32	0.1	0.10
Jul 25/72	24.5	---	7.9	400	0.24	0.1	0.13
Aug 3/72	24.0	---	8.0	400	0.18	0.1	0.10

* - ppm

∓ - no oxygen readings taken

TABLE 14 (continued).

Date	Total Coliforms Per 100 ml	Faecal Coliforms Per 100 ml
Sep 17/71	3.9×10^2	80
Sep 23/71	4.7×10^3	2.0×10^2
Sep 30/71	5.0×10^3	1.5×10^2
Oct 4/71	1.0×10^2	1.0×10^2
Oct 14/71	4.0×10^2	1.0×10^2
Oct 21/71	3.5×10^2	40
Oct 28/71	4.0×10^2	35
Nov 3/71	1.9×10^2	0
Nov 25/71	2.4×10^2	40
Dec 10/71	8.0×10^2	7.0×10^2
Dec 16/71	1.8×10^4	8.0×10^2
Jan 14/72	2.5×10^3	8.0×10^2
Jan 21/72	4.3×10^3	3.0×10^2
Apr 6/72	5.1×10^3	5.2×10^2
Apr 13/72	2.3×10^3	1.2×10^2
Apr 21/72	1.8×10^3	80
May 5/72	2.8×10^3	1.6×10^2
May 15/72	1.2×10^3	3.2×10^2
May 22/72	1.1×10^3	2.8×10^2
Jun 2/72	1.4×10^3	5.0×10^2
Jun 9/72	4.0×10^3	2.6×10^2
Jun 14/72	9.0×10^2	1.1×10^2
Jun 21/72	2.2×10^2	50
Jun 28/72	6.3×10^2	40
Jul 5/72	1.0×10^3	5.5×10^2
Jul 18/72	4.3×10^2	2.0×10^2
Jul 20/72	3.0×10^2	1.3×10^2
Jul 25/72	4.5×10^2	80
Aug 3/72	1.2×10^2	20

APPENDIX III

TABLE 15 - ANOVA OF THREE REPLICATES OF FAECAL AND TOTAL COLIFORM COUNTS FROM THREE PRIMARY SAMPLES COLLECTED JUNE 1, 1972 FROM SEWAGE INFLOW AND EFFLUENT.

Faecal Coliforms - Inflow

Source	df	SS	MS	F
total	8	20.00		
between	2	4.67	2.34	0.91
error	6	15.33	2.56	

Total Coliforms - Inflow

Source	df	SS	MS	F
total	8	38.89		
between	2	1.55	0.78	0.12
error	6	37.34	6.22	

Total Coliforms - Effluent

Source	df	SS	MS	F
total	8	712.22		
between	2	710.89	355.44	1615.66*
error	6	1.33	0.22	

P = 0.05 F significant at 5.14

* F significant

TABLE 16 - ANOVA OF THREE REPLICATES OF FAECAL AND TOTAL COLIFORM COUNTS FROM THREE PRIMARY SAMPLES COLLECTED MAY 23, 1972 FROM TWELVE MILE CREEK AND LAKE ONTARIO.

Feacal Coliforms - Twelve Mile Creek

Source	df	SS	MS	F
total	8	16.22		
between	2	0.88	.44	0.17
error	6	15.34	2.56	

Total Coliforms - Twelve Mile Creek

Source	df	SS	MS	F
total	8	1647.56		
between	2	107.00	53.50	0.21
error	6	1590.56	256.76	

Total Coliforms - Lake Ontario

Source	df	SS	MS	F
total	8	570.89		
between	2	140.22	70.11	0.98
error	6	430.67	71.78	

P = 0.05 F significant at 5.14

TABLE 17 - ANOVA OF THREE REPLICATES OF FAECAL AND TOTAL COLIFORM COUNTS FROM THREE PRIMARY SAMPLES COLLECTED JUNE 2, 1972 FROM TWENTY MILE CREEK.

Faecal Coliforms

Source	df	SS	MS	F
total	8	66.00		
between	2	0.23	0.12	0.01
error	6	65.77	10.96	

Total Coliforms

Source	df	SS	MS	F
total	8	62.00		
between	2	28.00	14.00	2.47
error	6	34.00	5.67	

P = 0.05 F significant at 5.14

APPENDIX IV

TABLE 18 - ANOVA FOR SEWAGE INFLOW AND EFFLUENT OF TOTAL AND FAECAL COLIFORM COUNTS.

Total Coliforms

Source	df	SS	MS	F
total	65	271.60×10^{12}		
between	1	70.60×10^{12}	70.60×10^{12}	2.2*
error	64	201.00×10^{12}	3.14×10^{12}	

$S_d = \sqrt{\frac{2S^2}{r}} = 4.36 \times 10^5$

$\bar{X}_1 - \bar{X}_2 = 1.67 \times 10^6$

$lsd_{(0.05)} = 2.14 \times 10^4$

Faecal Coliforms

Source	df	SS	MS	F
total	65	428.7×10^9		
between	1	55.3×10^9	55.3×10^9	9.5**
error	64	373.4×10^9	5.8×10^9	

$S_d = \sqrt{\frac{2S^2}{r}} = 1.88 \times 10^4$

$\bar{X}_1 - \bar{X}_2 = 5.8 \times 10^4$

$lsd_{(0.05)} = 3.8 \times 10^4$

P = 0.05 F significant at 1.8

* significant

** highly significant

TABLE 19 - ANOVA FOR SEWAGE EFFLUENT AND TWELVE MILE CREEK OF TOTAL AND FAECAL COLIFORM COUNTS.

Total Coliforms

Source	df	SS	MS	F
total	65	453.5×10^{11}		
between	1	30.6×10^{11}	30.6×10^{11}	4.64**
error	64	422.9×10^{11}	6.6×10^{11}	

$S_d = \sqrt{\frac{2S^2}{r}} = 2.0 \times 10^5$

$\bar{X}_1 - \bar{X}_2 = 4.18 \times 10^5$

$1sd_{(0.05)} = 4.0 \times 10^5$

Faecal Coliforms

Source	df	SS	MS	F
total	65	76.0×10^9		
between	1	6.6×10^9	6.6×10^9	6.0**
error	64	69.4×10^9	1.1×10^9	

$S_d = \sqrt{\frac{2S^2}{r}} = 8.1 \times 10^3$

$\bar{X}_1 - \bar{X}_2 = 2.0 \times 10^4$

$1sd_{(0.05)} = 1.62 \times 10^4$

P = 0.05 F significant at 2.0

** highly significant

TABLE 20 - ANOVA FOR TWELVE MILE CREEK AND LAKE ONTARIO OF TOTAL AND FAECAL COLIFORM COUNTS.

Total Coliforms

Source	df	SS	MS	F
total	69	89.77×10^9		
between	1	2.29×10^9	2.29×10^9	1.79*
error	68	87.48×10^9	1.29×10^9	

$S_d = \sqrt{\frac{2S^2}{r}} = 3.26 \times 10^3$

$\bar{X}_1 - \bar{X}_2 = 1.14 \times 10^4$

$lsd_{(0.05)} = 6.53 \times 10^3$

Faecal Coliforms

Source	df	SS	MS	F
total	69	10.57×10^5		
between	1	1.13×10^5	1.13×10^5	8.11**
error	68	9.44×10^5	0.14×10^5	

$S_d = \sqrt{\frac{2S^2}{r}} = 28$

$\bar{X}_1 - \bar{X}_2 = 80$

$lsd_{(0.05)} = 56$

P = 0.05 F significant at 1.76

* significant

** highly significant

APPENDIX V

In Figures 121-130 all coliform count data is presented in organisms per 100 ml while all nutrient and oxygen levels are given in parts per million.

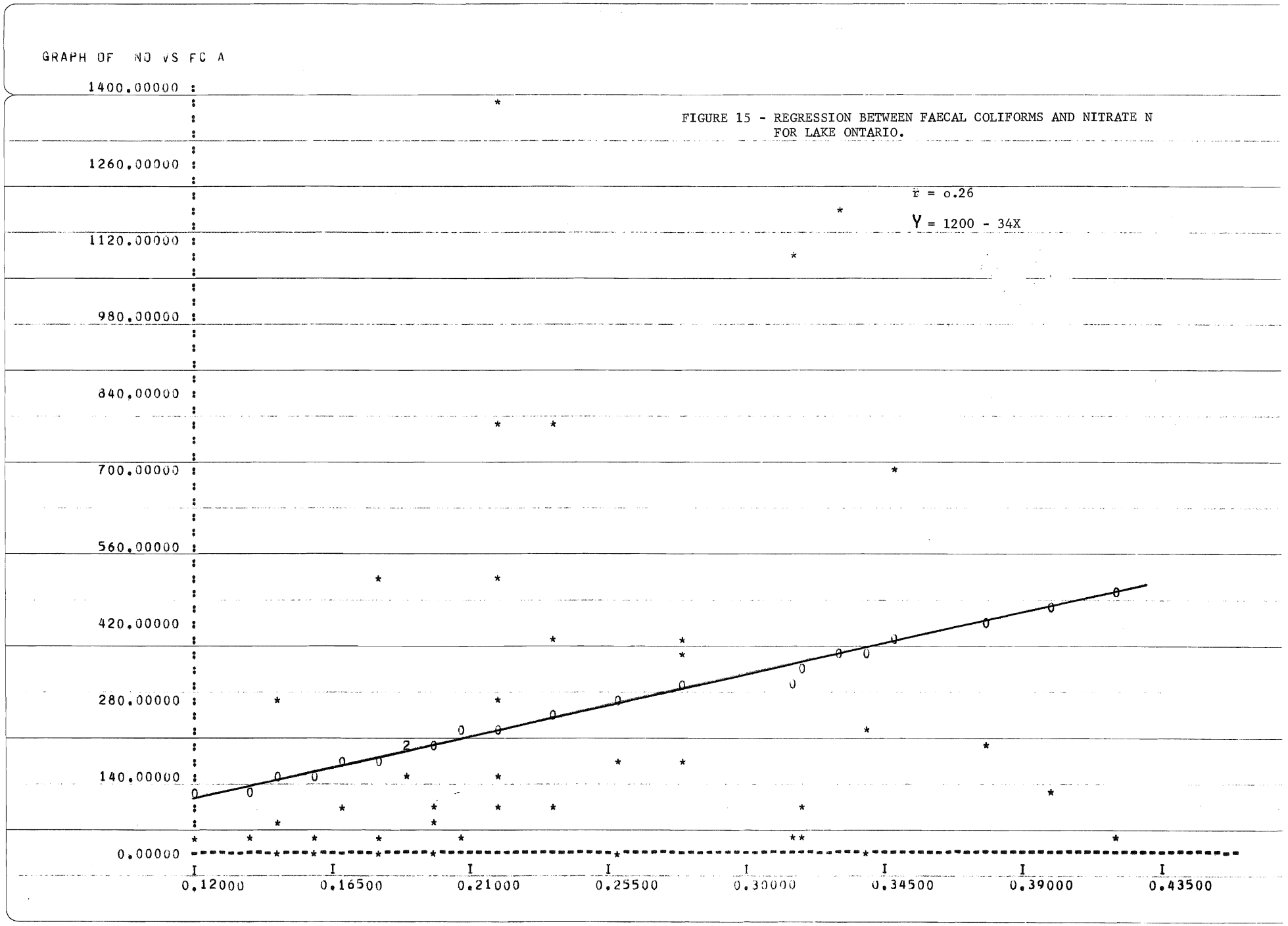


FIGURE 15 - REGRESSION BETWEEN FAECAL COLIFORMS AND NITRATE N FOR LAKE ONTARIO.

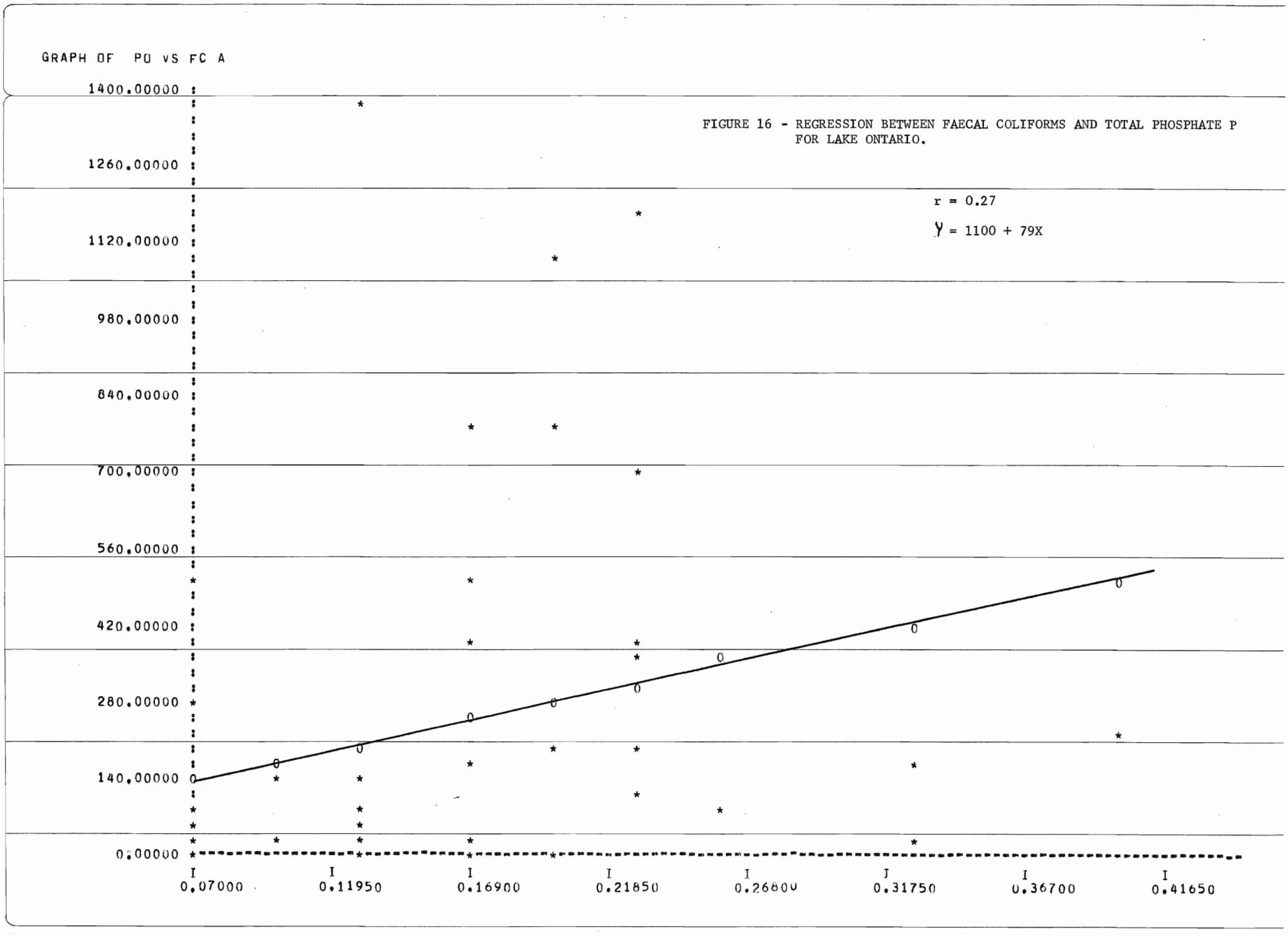


FIGURE 16 - REGRESSION BETWEEN FAECAL COLIFORMS AND TOTAL PHOSPHATE P FOR LAKE ONTARIO.

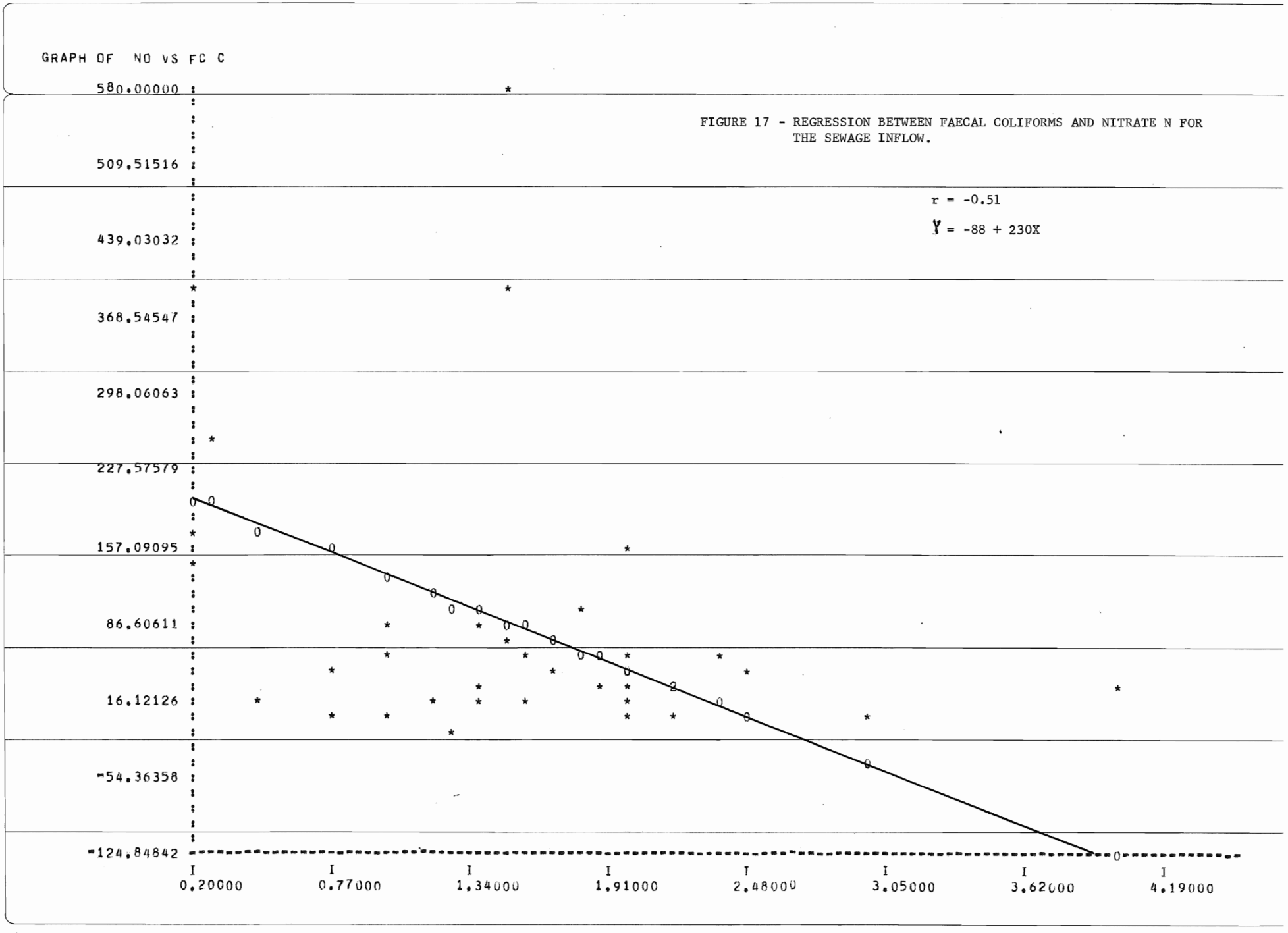


FIGURE 17 - REGRESSION BETWEEN FAECAL COLIFORMS AND NITRATE N FOR THE SEWAGE INFLOW.

$r = -0.51$

$Y = -88 + 230X$

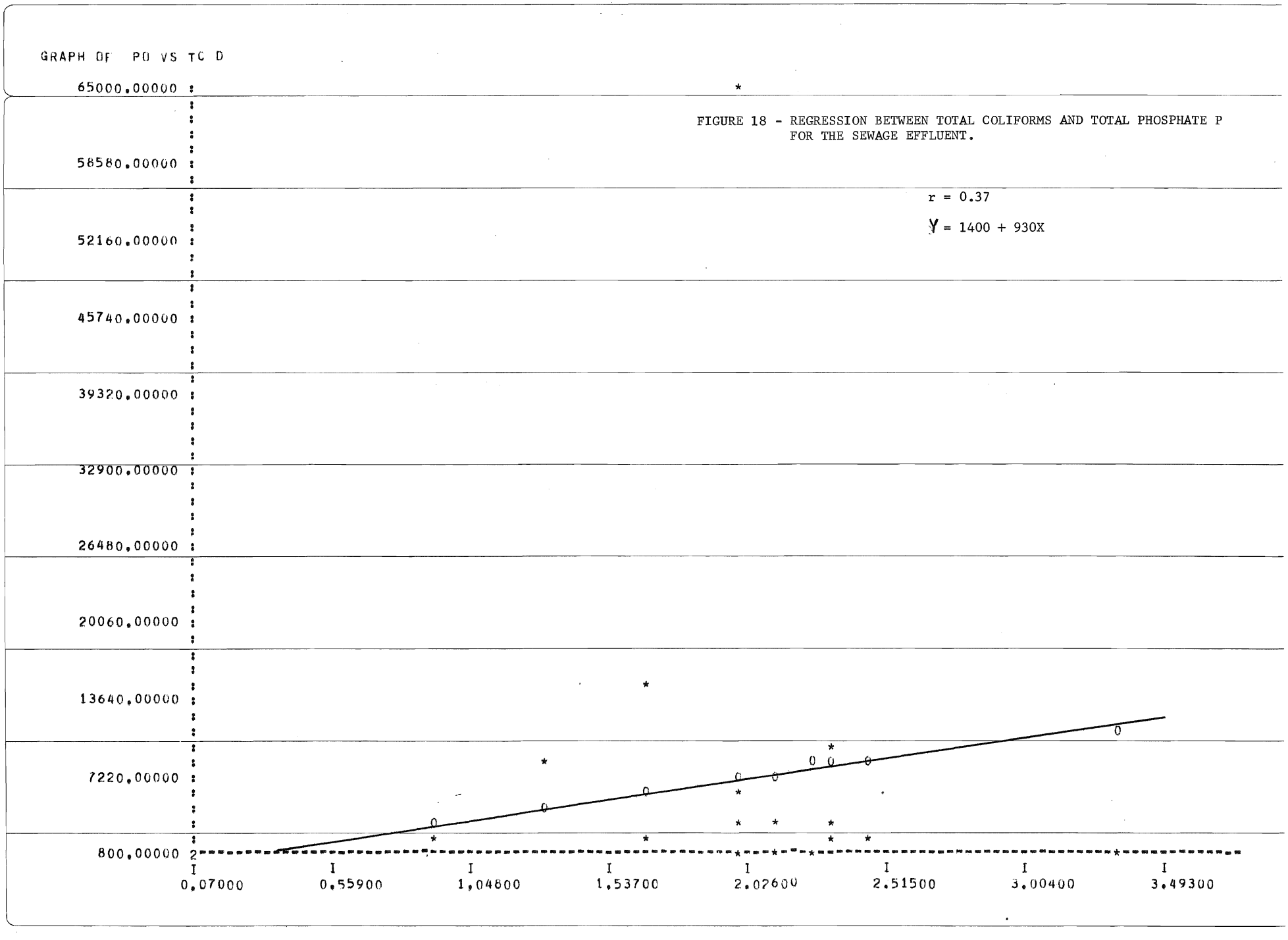


FIGURE 18 - REGRESSION BETWEEN TOTAL COLIFORMS AND TOTAL PHOSPHATE P FOR THE SEWAGE EFFLUENT.

$r = 0.37$

$Y = 1400 + 930X$

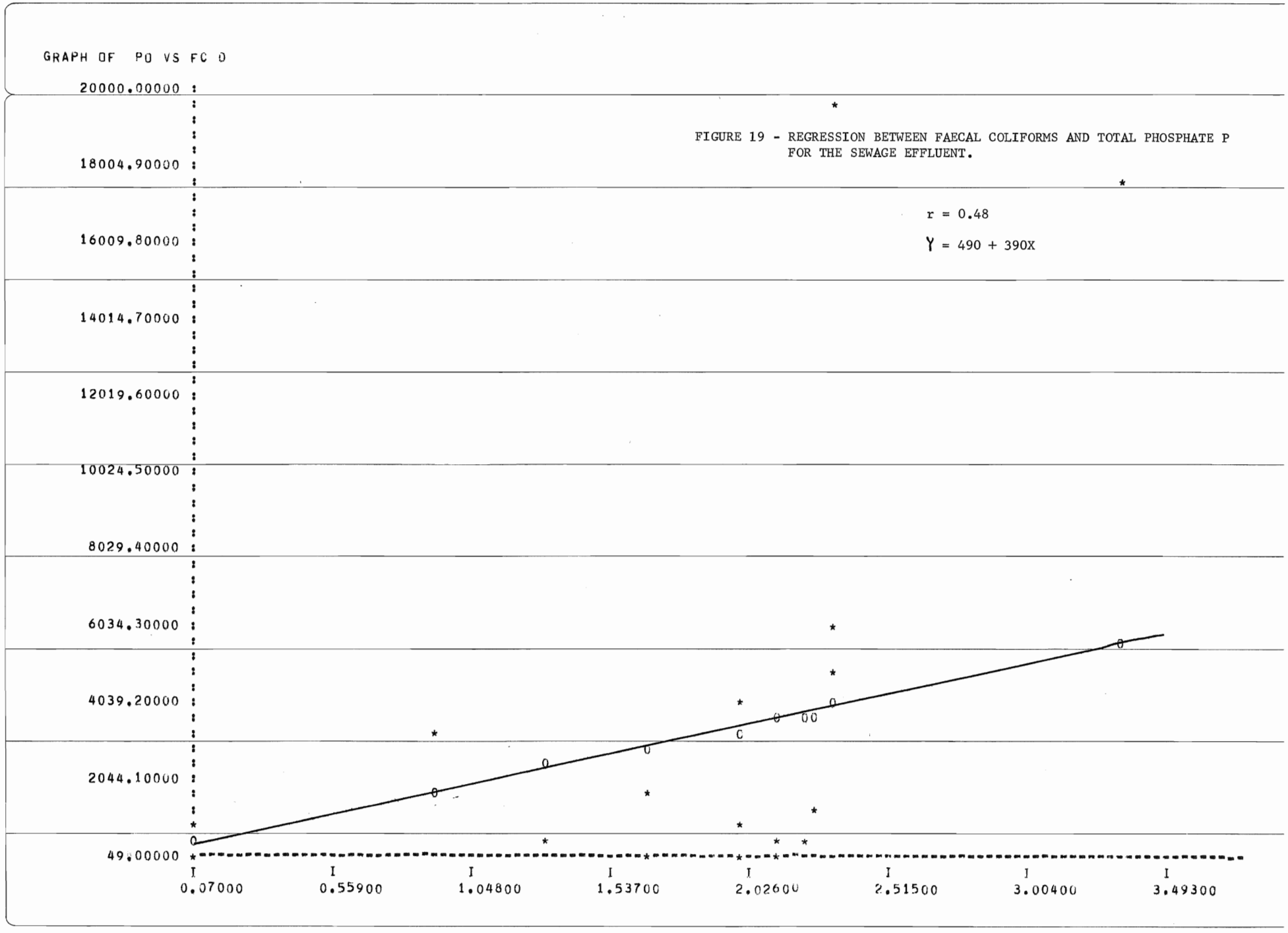


FIGURE 19 - REGRESSION BETWEEN FAECAL COLIFORMS AND TOTAL PHOSPHATE P FOR THE SEWAGE EFFLUENT.

$r = 0.48$

$Y = 490 + 390X$

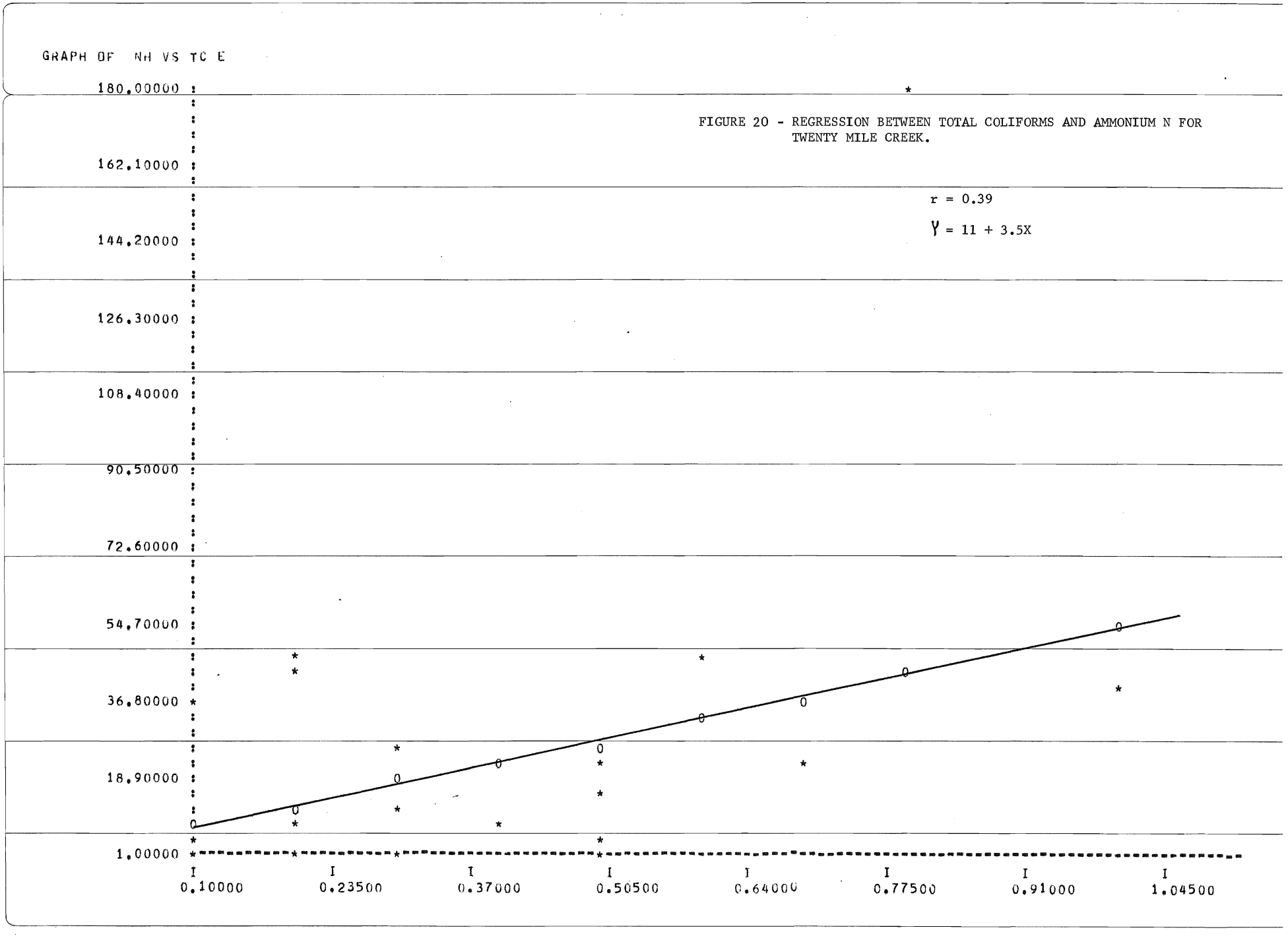


FIGURE 20 - REGRESSION BETWEEN TOTAL COLIFORMS AND AMMONIUM N FOR TWENTY MILE CREEK.

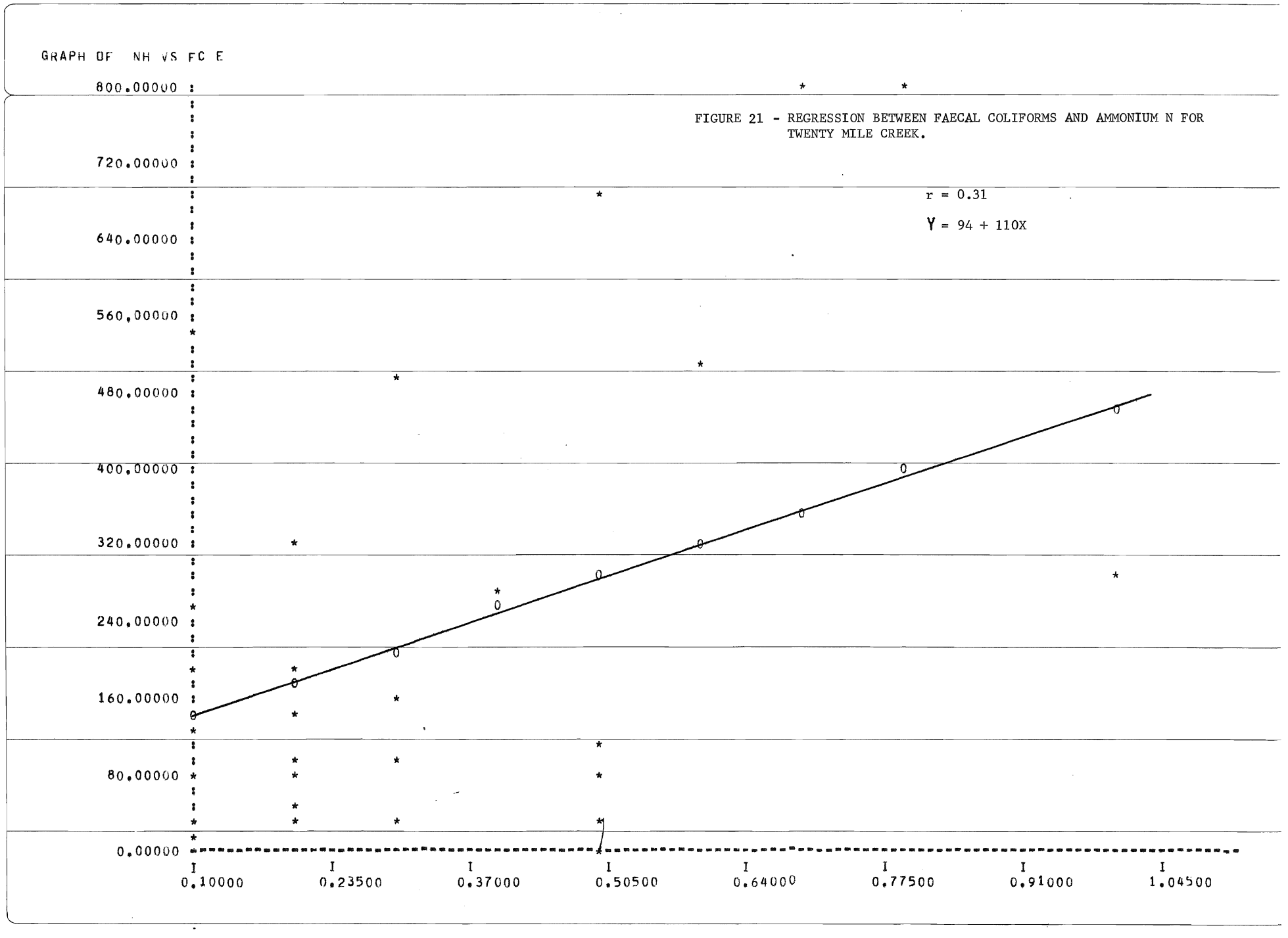


FIGURE 21 - REGRESSION BETWEEN FAECAL COLIFORMS AND AMMONIUM N FOR TWENTY MILE CREEK.

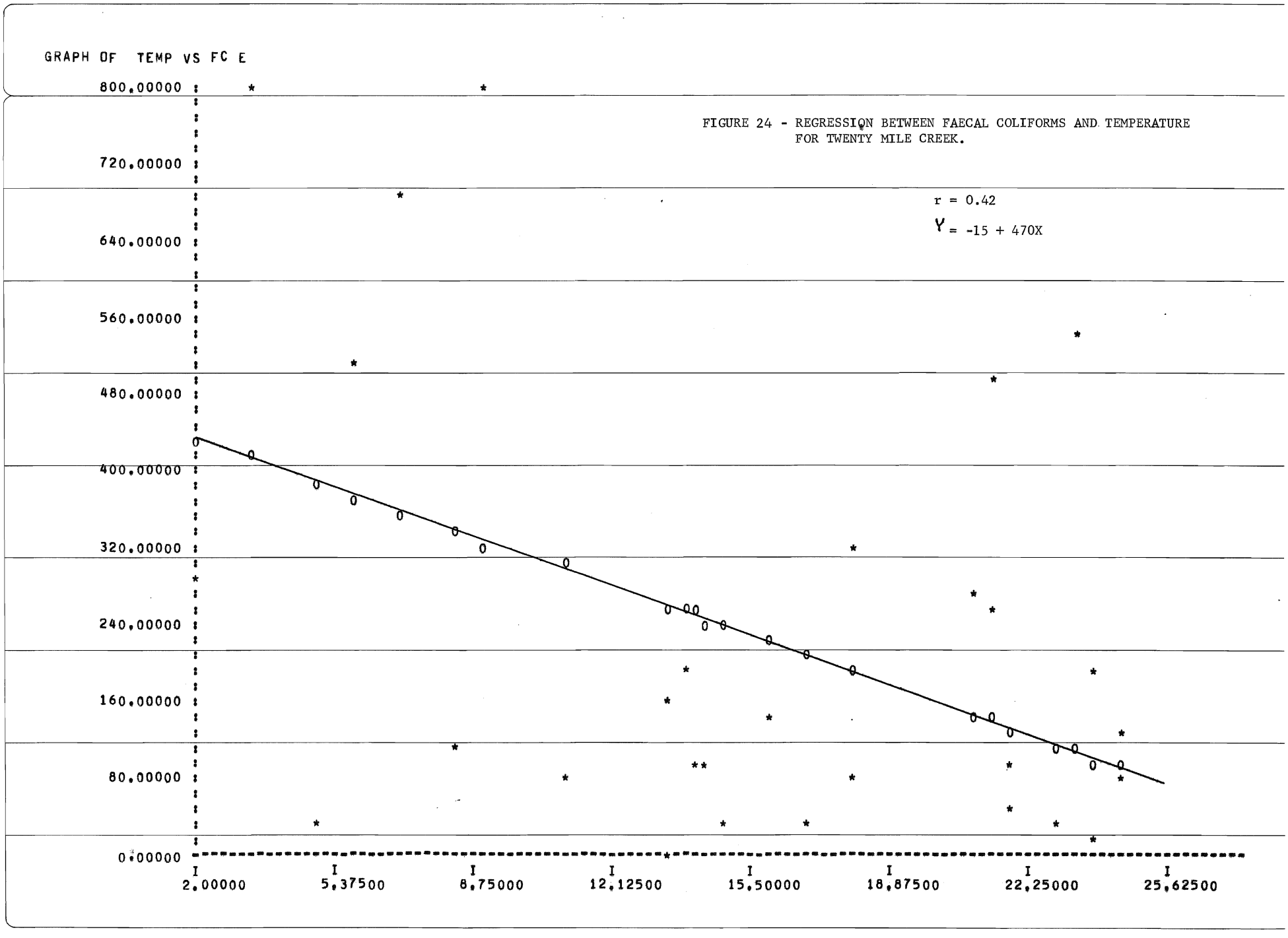


FIGURE 24 - REGRESSION BETWEEN FAECAL COLIFORMS AND TEMPERATURE FOR TWENTY MILE CREEK.

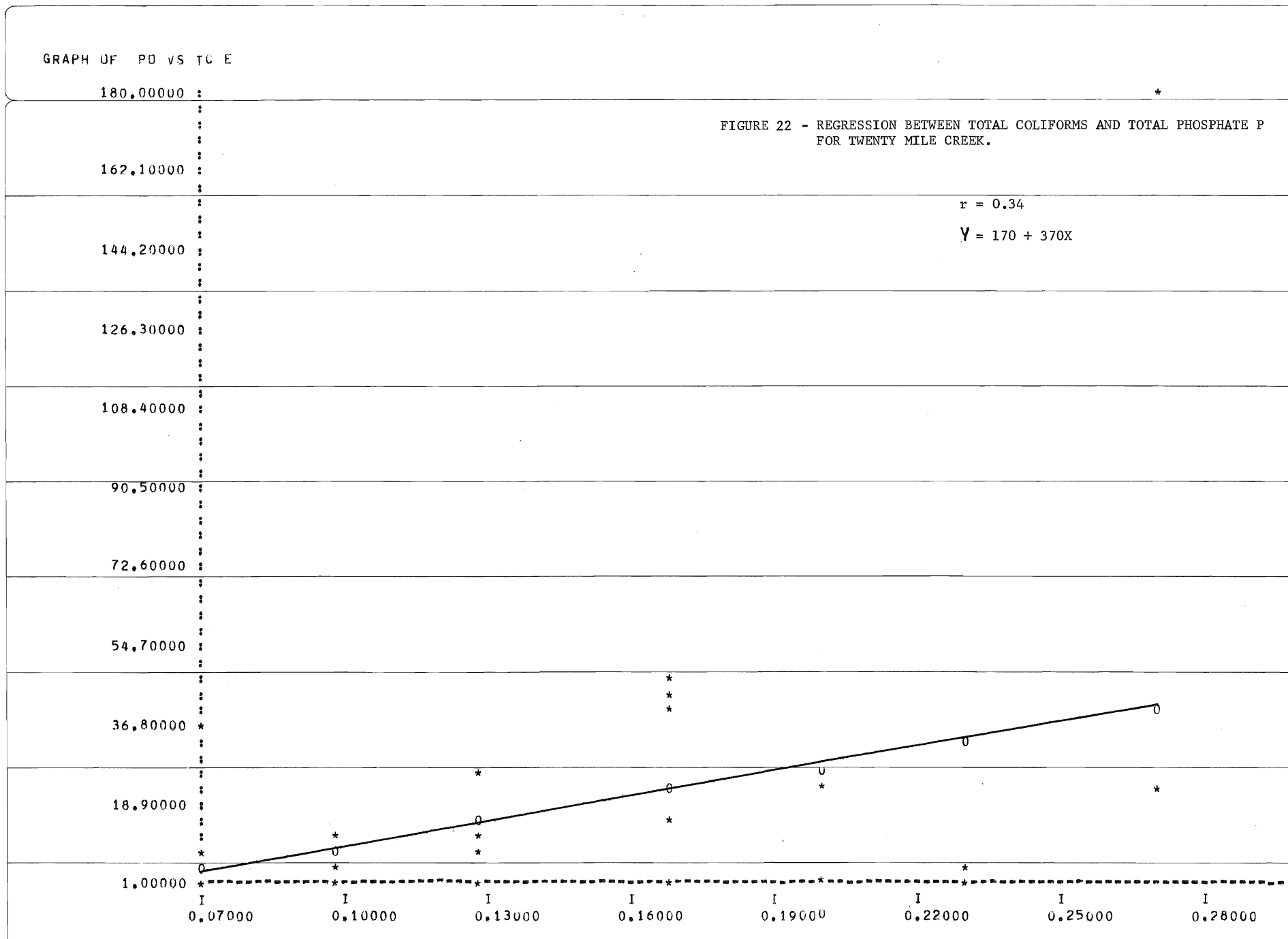


FIGURE 22 - REGRESSION BETWEEN TOTAL COLIFORMS AND TOTAL PHOSPHATE P FOR TWENTY MILE CREEK.

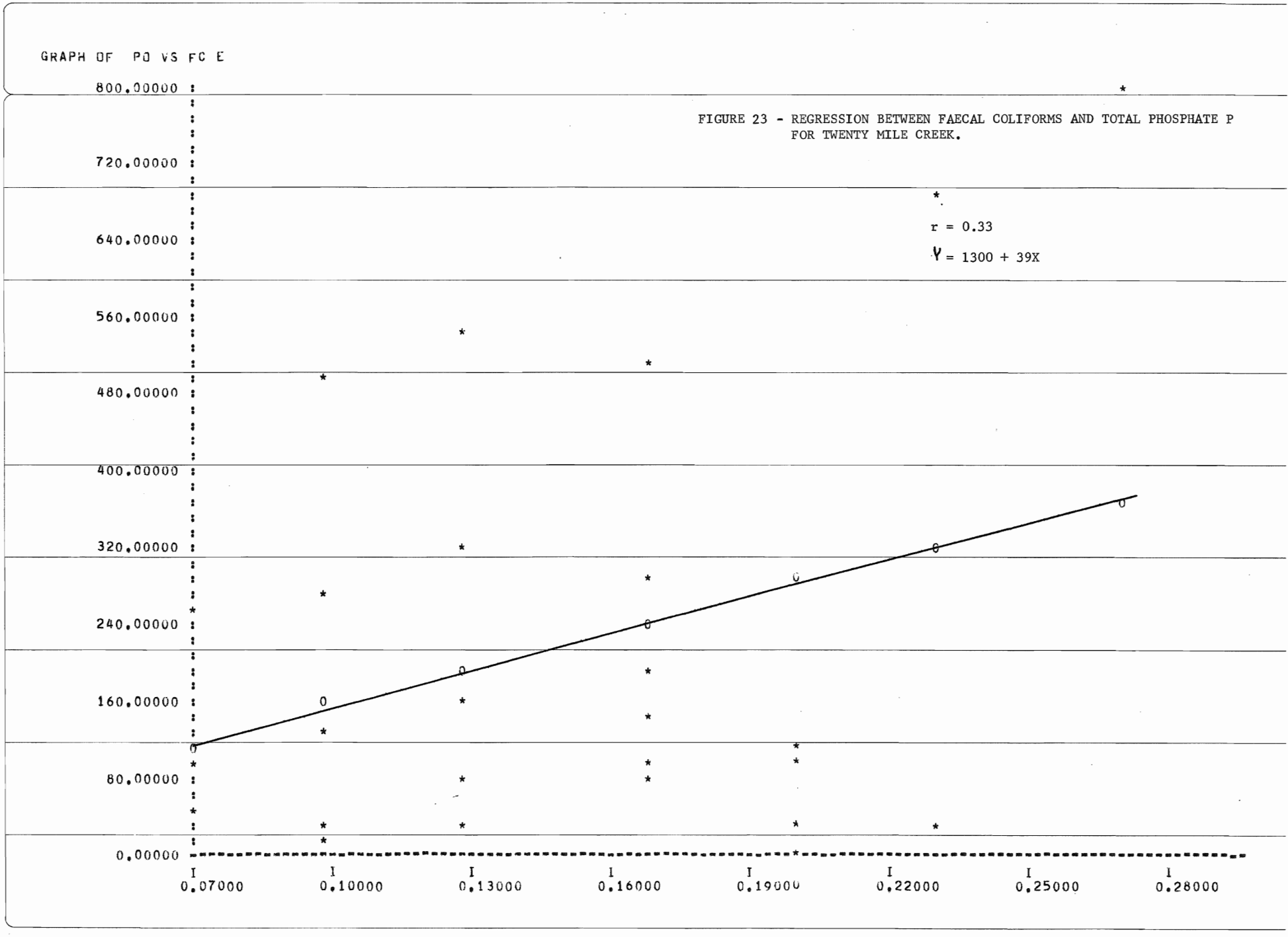


FIGURE 23 - REGRESSION BETWEEN FAECAL COLIFORMS AND TOTAL PHOSPHATE P FOR TWENTY MILE CREEK.